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Ultrasonic vocalizations (USVs) are commonly produced by many rodents, including all muroids investigated to date (18 genera). The overall adaptive significance of USVs within muroid rodents is not well understood. Most research has focused on the muroid genera *Mus* and *Rattus*. Within even these two relatively closely related genera, USV functions vary. Additionally, research on *Mus* and *Rattus* has been conducted exclusively in the laboratory and may be subject to laboratory effects. In order to contribute toward understanding the function of *Peromyscus* USVs, the context in which USVs are produced in the wild is investigated. Wild syntopic *Peromyscus californicus* and *P. boylii* are used as an example to explore 1) species differences in the spectral characteristics of USVs, and 2) interactions in USV production between two syntopic species. Both species vocalized, and the most commonly recorded USV motifs were 1-5 syllable vocalizations (SV). There are species differences in spectral characteristics of 1-5 SV USVs, but there is also high variability within each species. On average, *P. boylii* vocalizes 8 kHz higher than *P. californicus.* Frequencies do overlap between species, but frequency measurements can be used reliably to assign USVs to one of the two species, based on binary logistic regression and/or discriminant function analysis. Sixty-two percent of *P. californicus* and 82% of *P. boylii* USVs recorded occurred on the 42 nights (out of 123) when both species vocalized. Thirty-seven percent of *P. californicus* USVs and 52% of *P. boylii* USVs occurred within 5 minutes of an USV from a heterospecific. There were positive correlations between species in USV production on 8 out of 11 nights when each species produced more than 3 USVs, suggesting interactions between *P. californicus* and *P. boylii* do occur. Further research is warranted to understand the context and extent of the interactions.

SPECIES DIFFERENCES AND INTERSPECIFIC INTERACTIONS IN WILD *PEROMYSCUS* ULTRASONIC VOCALIZATIONS

by

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APPROVAL PAGE

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CHAPTER I

GENERAL INTRODUCTION

Ultrasonic vocalizations (USVs) are commonly produced by many rodents, including all muroids investigated to date (18 genera) [1]. The overall adaptive significance of USVs within muroid rodents is not well understood. Most research has focused on the muroid genera *Mus* and *Rattus* [1]. Within even these two relatively closely related genera, USV functions vary [2-11]. Additionally, research on *Mus* and *Rattus* has been conducted exclusively in the laboratory and may be subject to laboratory effects [1, 6, 12-15]. In order to contribute toward understanding the function of *Peromyscus* USVs, the context in which USVs are produced in the wild is investigated. Wild syntopic *Peromyscus californicu*s and *P. boylii* are used in this study as an example to explore 1) species differences in USVs, and 2) interactions in USV production between two syntopic species.

Rodents and USVs

Many small rodent species use ultrasound in social interactions, such as courtship, mating, aggression, territoriality, and alarms [14]. Ultrasonic rodent vocalizations are 20-100 kHz, and usually not longer than 300 ms, with bandwidths of 1-104 kHz, and intensities up to 103 dB in infants and 86 dB in adults [16]. In ground squirrels, USVs are used as alarms when predators are relatively far away, perhaps because the high frequency attenuates rapidly [17]. In

Family Muroidea, Order Rodentia (i.e. rats, mice, gerbils, etc.), USVs are known from several contexts, and seem to indicate affective states, sexual arousal, or to mediate social encounters.

Much of what is known about muroid behavior is due to the frequent use of *Mus* and *Rattus* as medical models in laboratory-based research. Laboratory rats and mice are known to use visual, chemical and acoustic communication [5, 18, 19]. Visual communication is used for close-range intention movements such as threat postures [18]. Chemical communication is likely used as a sexual isolation mechanism among sympatric species of mice [19]. All muroid infants that have been investigated are known to emit distress USVs in response to changes in the environment, perhaps to elicit maternal care [20]. Acoustic properties of infant USVs vary by emotional arousal, and response of adults to these USVs vary by adult demographic [20-22]. Adult USVs are different from infant vocalizations, spectrally and temporally [10].

The acoustic vocalizations of laboratory rats (*Rattus*) have been found to indicate affective states [3-9]. Fifty-kHz vocalizations indicate the appetitive or positive state, and 22-kHz vocalizations indicate the anxious, fearful or negative state [3-9]. Fifty-kHz vocalizations can even be elicited by tickling [23]. Female rats also emit USVs in response to male odor, and males emit 'mating' calls, though these calls are less important in mate attraction than the odor cues [21]. USVs have been useful in investigations of genetic and neurological mechanisms involved in emotional and neurobehavioral development [20, 24-31], and are also used as indicators in pharmacological studies of anxiety and memory [2, 27, 32]. USVs are not effective at indicating chronic pain, and instead are better at indicating transient states [12, 33-35]. There is evidence of individual differences in USV production in rats [33].

In laboratory mice (*Mus musculus*), USVs are 'encounter' calls, emitted in response to a novel social contact [2, 10], and are negatively correlated with 'social defeat,' that is, losing in a confrontation [35]. Males use USVs toward females, and females use USVs toward other females, especially those that may have access to desirable food resources [36]. Male 70 kHz vocalizations indicate sexual arousal [37]. Genotype also appears to have a role in the relative numbers of USVs produced, and in the use of USVs in inter- vs. intrasexual situations [38].

However, there are many confounding factors in the laboratory that prevent full understanding of USVs. For example, laboratory strains of rats and mice are inbred, artificially selected for a few phenotypic attributes; the effect this selection has on other aspects of the phenotype, such as calling behavior, could be vast [39]. Also, the animals are artificially contained in isolation or same-sex groups, which makes it hard to understand the possible social, intersexual, interspecific, or territorial functions or bases for these vocalizations. It is known that environment can affect behavioral results [15]. For this reason, it is important to study wild mice in their natural environment, where the adaptive significance can be unraveled and understood [6, 12-14].

Peromyscus

This study focuses on two species of *Peromyscus*, *P. californicus* and *P. boylii*. The genus *Peromyscus* (deer mice) includes over 50 species of deer mice, with ranges throughout North America [40]. Reproductive strategies range from obligate monogamy to promiscuity [40]. Ecological divergence between species appears to occur on the basis of microhabitat segregation [40]. However, microhabitat use is somewhat plastic and affected by various factors, including predation pressure, density, and darkness of nights [40]. Intra- and interspecific territoriality exists in some species at high densities (>25 mice per hectare) [40].

Peromyscus, including *P. californicus*, are used for reproductive, hormonal, and genetic studies [1, 41-45]. In laboratory studies, both infant [14, 22] and adult USVs have been recorded [M. Kalcounis-Rueppell, unpublished data].

The natural range of *P. californicus* is woodland habitat in coastal California [46]. Another species, *P. boylii*, occupies the same general habitat in the California mountains, and is active at the same time of night [46]. However, there appears to be habitat partitioning, whereby high density of *P. californicus* correlates to lower density of *P. boylii* within an area [46]. *P. boylii* is found more around live oak, while *P. californicus* is a habitat generalist [46]. *Peromyscus* are opportunistic feeders, and their diet ranges from nuts, seeds, insects, fungi to other vertebrates [47], but there is some food partitioning between these two species as well, due to size differences [46]. *P. californicus* is significantly larger than *P. boylii* [46].

Peromyscus boylii is promiscuous, with large male territories overlapping smaller female ranges [48]. *P. californicus* is biparental, monogamous, and aggressive [44]. In this species, females disperse more than males – female dispersal distance is affected by intrasexual competition, and male dispersal distance is affected only by resource competition [49]. Although interspecific territoriality has been shown within the genus *Peromyscus* [40, 50], it seems unlikely that this occurs between *P. californicus* and *P. boylii* because of the extent to which their home ranges and core areas can overlap [1, 50].

In 2005, 65 *Peromyscus* USVs were recorded in shared *P. californicus* and *P. boylii* habitat [1]. The recorded USVs fell into 7 distinguishable motifs [1], including multisyllabic vocalizations of 2-4 syllables. A “motif” is a stereotyped sequence of syllables [1]. A

"syllable" is a discrete, continuous sound, as viewed on a spectrograph, and separated from other sounds by a brief interval of silence (Fig. 1).

The difference in reproductive strategy and the shared habitat make *P. californicus* and *P. boylii* intriguing models for the study of USVs. For example, any species differences in USVs (whether based on sympatry, body size, or difference in reproductive strategy) should be readily apparent between these two species. Similarly, if there are interspecific interactions in USV production, simultaneous study of these two sympatric species should make it apparent. Therefore, this study expands upon the 2006 findings [1], using wild *Peromyscus californicus* and *P. boylii* as an example to explore 1) species differences in USVs, and 2) interactions in USV production between two syntopic species. Chapter II will focus on species differences in acoustic characteristics of USVs. Chapter III will focus on interspecific interactions in USV production. Chapter IV will summarize the findings of this study.

CHAPTER II

COMPARISON OF ACOUSTIC CHARACTERISTICS OF ULTRASONIC VOCALIZATIONS PRODUCED BY *PEROMYSCUS CALIFORNICUS* AND *PEROMYSCUS BOYLII*

Introduction

Communication is a transfer of information between sender and receiver, achieved through signals [51]. The benefits of communication via a signal must outweigh the energy requirements and the risks to the sender in order for the signal to evolve and persist [51]. To understand the adaptive significance of a signal, then, the benefits of using that signal must be understood. A primary step towards understanding the benefits, or function, of a signal is to determine the senders and receivers for that signal.

Species Differences

The simultaneous transmission of multiple signals between multiple sender and receiver groups, which will occur in a habitat, produces the need for a given receiver to be able to distinguish between personally relevant and irrelevant signals. Relevance can depend on such factors as species, group membership, or physiological condition, depending on the function of the signal. For example, because it is energetically important to mate only with conspecifics [52], and also energetically wasteful to defend territory from other species that do not use the same resources, some signals are solely intraspecific, particularly mating, courtship, and some

territorial signals [51]. These types of signals therefore evolve species-specific relevance information due to pressures from both sender and receiver. Specifically, it is detrimental to the sender to waste energy attracting or fighting irrelevant receivers, and it is detrimental to receivers to waste energy traveling toward or fighting irrelevant senders [51]. Receivers are therefore more likely to respond to signals that are clearly relevant [51]. Even closely related sympatric species become adept at differentiating between heterospecific and conspecific vocalizations, and will often ignore heterospecifics [53]. Species-specific elements within the signals allow for this relevance differentiation [53].

Likewise, if there is other information important to the relevance of the call, there should be group-specific elements within the signal to transmit that information, as well. There will be no selective pressure to include information not important to either the relevance or the message of the signal [51, 54]. Therefore, any information that is found to be included in the signal is likely important to the relevance or message. If the demographics (species, sex, age) of senders are known, comparison of signals across demographic groups for differences will allow determination of the elements within signals that may contain relevance information. Determining the demographic information embedded in a given signal is an important step toward understanding the likely receivers and/or function of that signal. For example, the discovery of species-specific elements within a signal would suggest that either the signal is an intraspecific signal, relevant only to conspecifics, or that species is in some other way important to the message or function of the signal.

Acoustic Communication

Specifically, acoustic communication involves the transmission and reception of sound waves over distance [51]. As sound waves travel through the environment, they are subject to

masking by other noises in the environment, both biotic and abiotic [52]. In order to reduce masking, signalers may altogether avoid frequencies already present in the environment, or call during quieter moments between the vocalizations of other species [14, 52]. Temporal partitioning in the production of acoustic signals has been shown to be an important method of denoting species relevance in some birds [55].

Within a signal, information – such as species, group, or individual identification, sexual receptivity or emotional arousal – can be contained in acoustic elements such as frequency, frequency modulation, amplitude, duration, temporal pattern, repetition period, energy distribution among harmonics, pitch interval between syllables, or sequencing of a call [10, 14, 56-58]. Frequency modulation and temporal patterning often contain species identification information [51, 58]. If most animals in a given habitat use the same or similar bands of frequencies due to environmental constraints on sound propagation, species identity information is often transmitted by temporal patterning added to signals [51]. Formant frequencies (frequency peaks) seem to be an honest signaling mechanism, correlating with demographic information such as age and size in many mammals, including humans and elephant seals [59]. The carrier or median frequency is more easily adjusted by the sender and may correlate more with emotional arousal [57, 59]. Amplitude can also be adjusted to some extent by the sender, and can be increased to reach a distant receiver [60].

Peromyscus

Species within the genus *Peromyscus* have been recorded producing ultrasonic vocalizations (USVs) in the wild [1]. The function of these signals is unknown. In fact, the function and adaptive significance of USVs is not well understood within the entire Muridae [1]. In order to begin understanding the function of USVs, it is important to determine which

demographic information these signals are transmitting [54, 57]. If there are species differences in USVs, then species is likely important to the relevance of the USV message.

Peromyscus is a particularly good muroid field model for studying species differences in USVs because of the local population density [40] and the extent of sympatry within this genus [40]. For instance, *P. californicus* and *P. boylii* live sympatrically in the coastal California mountains [1, 46]. There is some small-scale resource partitioning between species, but heterospecific home ranges extensively overlap [46, 47]. As species differences are typically particularly pronounced in sympatric species [52, 53, 61], any species differences in USVs within *Peromyscus* would be expected between coexisting *P. californicus* and *P. boylii*.

Objectives

The overall objective of this study was to examine the differences between *P. californicus* and *P. boylii* USVs. First, to determine whether any acoustic characteristics are significant species predictors, acoustic characteristics were examined within shared *P. californicus* and *P. boylii* motifs. Second, to determine the species classification success of USVs using acoustic characteristics as species predictors, statistical methods were used to assign USVs to species. Third, to determine any species differences in motif use, the within-species distribution of USV motifs was compared between species.

Methods

Data Collection

This study was approved by the Institutional Animal Care and Use Committee at UNCG. Data were collected from wild mice at Hastings Natural History Reservation in Carmel Valley, California (36° 22’ N, 121° 33’ W), February through June 2008. A remote microphone array,

telemetry system, and thermal imaging camera were set up to capture the natural nocturnal behavior of *Peromyscus*. The microphone array was used to record and localize *Peromyscus* USVs. The telemetry system was used to identify and locate individual *Peromyscus* in the area. The thermal imaging camera was used to record all activity within the area covered by the microphone array. Full details of data collection methods are below:

First, trapping for mice occurred on three established trapping grids, known as Lower Robinson Creek, Upper Robinson Creek, and Madrone Canyon (~3.75 ha total area), with trap stations spaced 10 m apart in a grid configuration (for details, see [46]). Two to four live traps were placed at each station. Both Sherman and Longworth traps were used. Traps were baited with a sunflower seed and rolled oats mixture, and contained a small amount of cotton bedding. All captured *P. boylii* and *P. californicus* were given ear tags, and basic data (age, sex, weight, reproductive status) were recorded for each individual.

Trapping data were used to map individual mouse locations in ESRI ArcView GIS 3.2. Mice captured at least three times within an area during trapping were defined as residents. Nine separate 10 m^2 'focal areas' were chosen in succession based on the number of residents, the presence of both species, and our ability to set up the microphone array and camera. In particular, vegetation and the presence of tall trees were important factors in focal area selection. At each of the successive focal areas, there was intensive trapping for three days to collar all residents with radio transmitters (0.55g M1450 from Advanced Telemetry Systems, each with a unique frequency, secured around the necks of the mice using fishing line and plastic tubing). Up to 5 traps were placed at nearby trapping stations and up to 16 traps were placed within the focal area, and were checked twice per night, in order to ensure that all resident mice were captured. If all

residents were not captured within 3 days, trapping was stopped for a few days and then resumed for 3 more nights.

At the focal area, a 4x3 grid (approximately 10 m^2) of twelve Emkay FG Series microphones was set up on the ground, and connected through an Avisoft UltraSoundGate system (Avisoft Bioacoustics) to a laptop (DELL Latitude D410) running Avisoft RECORDER software. Using this software, each microphone recorded a separate sound wave within one sound file, each time that the software was activated by sound. The sound file opens as a spectrographic array on Avisoft SAS-Lab PRO, showing a separate spectrogram from each microphone. The time (to the second) that each sound file was recorded was automatically included in the sound file name.

Four Sigflex 15 cm omni-directional antennae were set up – one antenna set at each corner of the microphone grid – and connected to an Advanced Telemetry Systems 4MHz R4000 radio signal receiver. The receiver was placed within a metal trashcan (acting as a Faraday Box) to ensure that all signals were received exclusively through the four antennae. The receiver was set to scan for all resident transmitter frequencies, and was attached to an Advanced Telemetry Systems DSU D50410 data logger to store data on received signals. When a signal was detected, data from all four antennae was recorded. Logged data included: time the signal was received (to the minute), transmitter frequency, antenna where signal was received, and signal strength.

A thermal imaging lens (Photon 320 14.25 mm; Flir/Core by Indigo) was suspended in the forest canopy above the focal area, using ropes and pulleys attached to neighboring trees, at approximately 30 feet above the ground, so that the lens view captured the entire 10 m^2 focal area. This lens was attached to a JVC Everio HDD camcorder with a 30 GB hard drive to store the video footage. The continuous footage was broken into a number of separate video files by

the camcorder. The time display on the camcorder recorded the time (to the minute) when each video file began.

Clocks on the laptop, telemetry data logger, and camcorder were synchronized daily. Each was connected to 12 V 33 Amp Hour car batteries via inverters for power. Batteries were recharged daily. The data collection equipment was generally set out at about 5 PM and collected at 5 AM the following morning. Each day when equipment was collected, data were downloaded from laptop, data logger, and camcorder to DROBO (Data Robotics, Inc) external hard drives. Data were daily examined to ensure that equipment was working properly and to evaluate data collected. The three systems (microphones, telemetry, camera) remained in place for about three weeks to record vocalizations, location of collared individuals, and general activity within the focal area. Once the majority of transmitters were no longer giving good signals (~ 2-3 weeks), intensive trapping was resumed to remove the collars. The three-system setup was then moved to a subsequent focal area.

Analysis of Remotely Collected Data

Because the Avisoft RECORDER software was set to be sound-activated, and was activated by a variety of noises, many of the recorded sound files did not contain *Peromyscus* USVs. In order to separate out the relevant sound files, all the files were visually examined via the spectrograph array on Avisoft SASLab-Pro software for similarity to known *Peromyscus* USVs. All sound files showing potential USVs were examined visually and acoustically to eliminate "non-biological" sounds (i.e., static, mechanical, rain, or movement sounds). Sounds of bats and birds were eliminated based upon shape of spectrograph, frequency, and playback of sound. Sound was played back at 4.4% of normal speed (speed reduced by a factor of 22) and/or at normal speed. Once all sound files were examined and categorized into specific USV motifs,

all files that were determined to have come from a mouse were subject to the following analysis to determine which individual mouse made the sound.

First, every USV sound file was subjected to visual spectrographic array analysis to determine the order of sound arrival at the microphones in the focal area grid. The USVs were visible in spectrographs from each individual microphone in the array, and by comparing the arrival time of an USV at a microphone to the arrival time at other microphones, it was possible to determine the order in which the USV had arrived at microphones within the array. Using a diagram of the numbered microphone array, an estimate was made of where on the focal area the sound originated (Fig. 2). For example, if a sound arrived at four microphones which make up a square of the grid before arriving elsewhere, the sound was estimated to have come from within that square. If sound arrived at all microphones along one side of the grid before arriving at interior microphones, the sound was estimated to have come from outside the grid, on that side. If sound arrived first at a corner microphone and arrived at surrounding microphones in a diagonally spreading fashion, the sound was estimated to have come from that corner (either inside or outside the focal area grid).

Secondly, telemetry data files were made of the telemetry data logged in the ten minutes surrounding each USV sound file (5 minutes on either side), from which the species/individuals present at the time of the recording were determined. Distances between a detected mouse and the antenna where it was detected were estimated based on the signal strength logged. In order to estimate the relationship between signal strength and distance, two types of transmitter/receiver tests were conducted. First, four transmitters were tested at 1 m intervals (1-9 m) from each antenna of the telemetry system 24 times. The relationship between signal strength and distance was determined via linear regression for these four transmitters (see Appendix A). Because ANOVA showed differences between transmitters in these data, transmitter tests were done for

each transmitter, prior to its use, at the appropriate focal area. For these tests, each transmitter was placed at each microphone within the focal area. The known distance between each microphone and antenna, along with the logged signal strength, was used to produce a linear regression and distance prediction interval for each transmitter used (see Appendix A). Using the telemetry block for a given call, and the distance prediction intervals for each transmitter detected, an estimate of the location of each detected mouse during the call was made.

Next, video clips were made of the thermal images during the minute when each USV sound file was recorded, using Cyberlink PowerDirector editing software. Since the start time of each video file was shown only to the minute, video and sound files could not be synchronized to the exact second when a USV was recorded. Therefore, a full one minute clip of the video was made, encompassing the entire time during which the sound file could have been recorded. These video clips were viewed on Windows Media Player and notes were made of any activity within the field of view. Noted activity included all on-screen movement, as well as the number of on-screen mice (both moving and stationary).

All sound file, telemetry, and video data were organized within a Microsoft Excel spreadsheet so that, for each USV sound file, notes on the location of USV sound origination, location of mice within the focal area, and video-recorded activity on the focal area were viewable at once. Using these data, three separate observers assigned each USV to an individual mouse (by transmitter frequency/ear tag number). This process was referred to as an overlay. The overlay process was separately completed by three different observers to ensure that subjectivity was minimized in the assignment of vocalizations to individuals. USV assignments which were disagreed upon were revisited by a single observer. All USVs assigned to individuals in this manner comprised the dataset hereafter referred to as Overlay Vocalizations. The remaining

USVs, which could not be assigned to an individual by the overlay process, comprised the dataset hereafter referred to as Classified Vocalizations. The dataset All Vocalizations includes both Overlay Vocalizations and Classified Vocalizations.

Spectrographic Analysis of USVs

All *Peromyscus* USVs were analyzed via Automatic Parameter Analysis in Avisoft SASLab-Pro for 14 variables per syllable. The duration of each syllable was measured, as well as the minimum frequency, maximum frequency, peak frequency and bandwidth at the start of the syllable, the end of the syllable and the time of maximum amplitude of the syllable. Group duration (duration of the entire call), total bandwidth, and interval duration for each interval (between syllables) in the call were also measured. Parameters were measured from a spectrograph with an FFT length of 256 and a frequency range of 125 kHz in a Hamming window with 100% frame size. Only one spectrograph per USV was used for analysis. The spectrograph was chosen based on amplitude and clarity of the USV. Background noise was erased from the spectrograph where necessary to ensure that the parameter measurement cursors measured only the USV in question. Automatic Parameter Analysis results for each USV were copied and pasted into a Microsoft Excel spreadsheet. Parameter analysis was completed by two different observers, but a subset of the USVs was analyzed by both observers, and a paired t-test compared each variable measurement for each of these USVs to determine whether there were significant differences between observers in cursor placement and subsequent parameter measurement. A paired t-test for each measured variable in 52 randomly chosen USVs showed no significant difference any of the variable measurements between the two observers who performed parameter analysis on Avisoft SASLab-Pro ($p>.05$ for all variables).

Statistical Analysis

Objective 1. Using Overlay Vocalizations, forward stepwise binary logistic regression on SPSS 16.0 was used to determine which of the variables (14 per syllable plus group/interval measurements) were significant predictors of species. Inspection of box plots of significant predictors aided in predictor selection. Each motif was analyzed separately due to the difference in number of syllables (and therefore variables). The significance of the variables as species predictors was quantified by the stepwise logistic regression. The actual unit differences between species in the chosen variables were quantified by a comparison of means. The use of Principal Components rather than individual variable measurements was also attempted, but better classification success of Overlay Vocalizations was achieved using the original individually measured variables.

Objective 2. Overlay Vocalizations were assigned to *P. boylii* or *P. californicus* via binary logistic regression and discriminant function analysis on SPSS based on USV spectral measurements, using the variables found to be the best species predictors for each motif. Species predictors used for classification were chosen based on highest correct classification of Overlay Vocalizations. Variables differed slightly from those used in Objective 1 because variable selection was based entirely on classification success of Overlay Vocalizations. When fewer predictors had better or equal success in classification, fewer predictors were used. The same variables were used as group predictors in both the logistic regression and discriminant function analysis. Predicted group membership and probabilities of correct group membership were listed for each USV by both binary logistic regression and discriminant function analysis. Where these methods did not agree, the method that showed the highest probability for correct classification of that call was used. Where probabilities of correct classification were similar, the group

membership assignment by logistic regression was used, as the data did not show normal distribution. In order to expand the dataset for further analyses, Classified Vocalizations, which were not assigned to individuals via the overlay process due to lack of clarity in the data, were assigned to species via this statistical method.

Objective 3. Overlay Vocalizations were listed by species, and the number of vocalizations of each motif was counted for each species, to determine whether there were any motifs exclusive to one species, or whether there were any motifs more common to one species than the other. All Vocalizations was also tested by chi-square analysis to determine whether there was any difference in distribution of motifs between species within this much larger dataset. Motif distribution was also compared between Overlay Vocalizations and Classified Vocalizations by chi square analysis to determine whether there were significant differences in motif distribution between the two data subsets. Chi-square analysis was conducted on Microsoft Excel 2007.

Results

Data was collected from 9 focal areas, each with 4-13 residents (avg per focal area = 7.22, SD= 2.91). All focal areas contained overlapping home ranges from both species. Among 109,021 sound files recorded over 123 nights, 1090 *Peromyscus* USVs were found. 1-5 syllable vocalizations (SV) accounted for 1050 of the *Peromyscus* USVs. The remaining 40 USVs were distributed among 4 other motifs. Due to difficulties assigning these 40 USVs to individuals, and the overall rarity of the motifs, they were eliminated from the dataset for the purposes of this study. The 1050 1-5 SVs were produced on 95 out of the 123 nights.

Out of the 1050 1-5 SVs recorded, 246 were assigned to an individual mouse with full initial agreement from all three observers. Of the remaining USVs, 147 were assigned to

individuals after reinspection of the data. It was agreed that 621 USVs could not be assigned to an individual due to inadequate resolution in the telemetry data and/or failing transmitters. In the end, 393 USVs were assigned to individuals via the overlay method. These 393 USVs comprised the Overlay Vocalizations. Of these, 232 were assigned to *P. californicus* and 161 were assigned to *P. boylii*. The remaining 657 1-5 SV USVs (Classified Vocalizations) were assigned to species based on binary logistic regression and discriminant function analysis using the chosen species predictor variables for each motif. All 1050 1-5 SVs were included within the All Vocalizations dataset.

Objective 1. Using only Overlay Vocalizations, spectral characters distinguished between species for all motifs.

Significant species predictors found by stepwise binary logistic regression of 1-SV were the minimum frequency at the end of the call and duration (model χ^2 stat=27.76, p<.01, n=80, R^2=.39). *Peromyscus boylii* 1-SVs had a higher frequency and were longer than *P. californicus* 1-SVs (Table 1).

For 2-SVs, the most significant species predictors were the minimum frequency at the point of maximum amplitude of the second syllable, and bandwidth at the point of maximum amplitude in the first syllable (model χ^2 stat= 60.05, p<.01, n=148, R^2=.45). *Peromyscus boylii* vocalizations were again higher than *P. californicus*, with a smaller bandwidth (Table 2).

For 3-SVs, the most significant species predictors were the minimum frequency at the point of maximum amplitude of the second syllable, duration of the third syllable, and bandwidth at the point of maximum amplitude of the first syllable (model χ^2 stat=80.36, p<.01, n=117, R^2=.69). *Peromyscus boylii* vocalizations were higher, with longer duration and smaller bandwidth than *P. californicus* (Table 3).

For 4-SVs, the most significant species predictors were the maximum frequency at the end of the fourth syllable and the bandwidth at the end of the third syllable (model χ^2 stat=30.74, p<.01, n=40, R^2=.76). *Peromyscus boylii* vocalizations were higher with a larger bandwidth than *P. californicus* (Table 4).

For 5-SVs, due to very small sample size, all frequency variables were highly significant (model χ^2 stat=9.00, p=.003, n=8, R^2=1). *Peromyscus boylii* vocalizations were higher in frequency than *P. californicus* (Table 5). Due to the small sample size of 5-SV, the number of predictors entered into the stepwise logistic regression exceeded the sample size, resulting in the R-square value of 1.

Objective 2. There was an overall 81% success rate in correctly classifying Overlay Vocalizations to species using statistical methods based on acoustic measurements of USVs, using binary logistic regression and discriminant function analysis together. There was an overall 92% agreement between binary logistic regression and discriminant function analysis in USV assignments. Species predictors used for classification of 1-SV were the minimum frequency at the point of maximum amplitude and duration (model χ^2 stat=25.07, p<.01; Table 6). The species predictor used for classification of 2-SV was the minimum frequency at the point of maximum amplitude of the second syllable (model χ^2 stat=48.63, p<.01; Table 6). Species predictors used for classification of 3-SV were the minimum frequency at the point of maximum amplitude of the second syllable and bandwidth at the point of maximum amplitude of the first syllable (model χ^2 stat=76.30, p<.01; Table 6). Species predictors used for 4-SV classification were the maximum frequency at the end of the fourth syllable and the bandwidth at the end of the third syllable (model χ^2 stat=30.74, p<.01; Table 6). The species predictor used for classification of 5-SV was the minimum frequency at the point of maximum amplitude of the first syllable (model χ^2

stat=9.00, p=.003; Table 6). All 657 Classified Vocalizations were assigned to a species using this statistical method.

Objective 3. Both species produced all five motifs represented by 1-5 SV USVs (Fig. 3). The most commonly used motifs by both species were 1-3 SV (Fig. 3). Chi-square analysis showed homogeneity between species among the counts of the 5 motif types within the Overlay Vocalizations (χ2= 6.42, df= 4, p=.17). Within All Vocalizations, there was a significant difference between species (χ2= 22.19, df= 4, p<.01), with *P. boylii* producing more 2-SV and *P. californicus* producing more 1-SV. A chi-square analysis of the proportional motif distribution in Overlay Vocalizations versus the Classified Vocalizations showed a significant difference (χ^2= 52.32, df= 4, p<.01), with a disproportionate number of 1-SV within Classified Vocalizations and a disproportionate number of 3- and 4-SV within the Overlay Vocalizations.

Discussion

There are species differences in spectral characteristics of 1-5 SV USVs. However, there is also variability within each species, as reflected in Tables 1-5. Because there is only slight frequency modulation within 1-5 SV USVs, the patterns shown in the frequency variables used for species differentiation are also found in the other frequency variables of that syllable. For all motifs, *P. boylii* vocalize at a significantly higher average frequency than *P. californicus*. Duration is also a significant species predictor for 1-SV. Bandwidth is a nonsignificant but helpful species predictor for 4-SV. Additionally, USVs can reliably be assigned to species via statistical methods based on spectral characteristics. Again, spectral measurements which provide the best classification success are primarily frequency measurements.

Because there are spectral differences between species in 1-5 SV USVs, I tentatively propose that species identity is an important part of the USV message. This may be to indicate to

conspecifics that a signal is relevant (where a heterospecific's is not) or may be to indicate species (as a relevant part of the USV message) to heterospecifics.

However, the species differences revolve primarily around frequency. As mentioned previously, other research has shown that species identification information is most often communicated through temporal patterning and/or frequency modulation (total bandwidth of the call) [52, 59]. And, though there are significant differences in the average frequencies of *P. californicus* and *P. boylii* USVs, and vocalizations can be readily categorized by species based on this, the within-species variation is so high that the frequencies do overlap. It is possible that the species difference in frequency is due solely to body size differences (*P. californicus* ~ 40 g, *P. boylii* ~ 30 g), and that species is not in fact an important part of the USV message. It is very difficult for small animals to produce low frequencies, because of the size of low frequency sound waves [52]. And, the larger the wavelengths are relative to the size of the animal, the lower the intensity of the sound emitted [52]. Therefore, acoustic frequency is often inversely correlated to body size [52, 63]. Further research into the relation of body size to USV frequency within *Peromyscus* will help clarify this issue. Comparison of USVs between similarly sized wild *Peromyscus* species may be particularly useful.

Further experimentation can also be done do determine whether species identification information is important to USV relevance. For example, experimental observation of *P. californicus* behavior in the presence of other *Peromyscus* species' USV playbacks would help determine whether species identification information is important to USV relevance in *P. californicus*. If the response is different to heterospecifics and conspecifics, then species can reliably be said to be important to USV relevance in *P. californicus* [53]. Likewise, if playback experiments show no difference in response to heterospecifics and conspecifics, it would solidify

the argument that species information is not generally important to USV relevance in *Peromyscus*. Testing the response to playbacks also allows understanding of possible species divergence in the perception of USVs [52, 54].

None of the 1-5 SV motifs are used exclusively by one species, but *P. boylii* produced more 2-SV and fewer 1-SV than *P. californicus*. The motifs most commonly used by both species are 1-3 SV, and 2-SV are the most common overall for both species (only slightly more common than 1-SV in *P. californicus*). The discrepancy in motif distribution significance between Overlay Vocalizations and All Vocalizations is due to the difference in motif distribution between the Overlay Vocalizations and Classified Vocalizations data subsets. Because the sample size is much larger when the Classified Vocalizations are added to the dataset, and because similar nonsignificant trends in within-species motif distribution are seen in the Overlay Vocalizations, it is expected that the analysis of the larger dataset is more accurate, and that there is a significant difference in motif distribution between species.

While there may be significant differences in the proportional use of motifs by species, both species produce all 5 motifs, and therefore motif is not in any way a reliable species indicator. Laboratory data support this finding, as 1-4 SV are commonly recorded from all studied *Peromyscus* species [M. Kalcounis-Rueppell, unpublished data]. Motif may be important to some other aspect of the USV message, possibly indicating other demographic relevance or a graded affective state, such as urgency. Further research into motif use will help clarify the significance of the species difference in motif distribution.

Overall, the data from this current study tentatively suggest that species identity is important to USV relevance and/or message in sympatric wild *Peromyscus*. This may be a

response specifically to sympatry or may be the result of USVs diverging in concert with the adaptive radiation of *Peromyscus*. Comparisons of allopatric *Peromyscus* USVs could shed light on this question. If sympatric USVs are more different than allopatric USVs, then sympatry must be a driving force in the development of species differences [54]. If sympatric USVs are not more different than allopatric USVs, species differences may be an artifact or even a mechanism of speciation [64] in *Peromyscus.*

Further research into species differences in USV spectral characters is certainly warranted, both within *Peromyscus* and within Muridae. Study of additional species is necessary to produce conclusive results regarding the importance of species and/or genus to the relevance or function of USVs.

CHAPTER III

INTERSPECIFIC INTERACTIONS IN ULTRASONIC VOCALIZATION PRODUCTION BETWEEN *PEROMYSCUS CALIFORNICUS* AND *PEROMYSCUS BOYLII*

Introduction

Known interspecific communication primarily revolves around alarm calls, distress calls, anti-predator exhibitions of vigor, and occasionally 'resource-recruitment' signals when anti-predator and mass foraging benefits outweigh food competition losses [51, 62]. The most demonstrated example of an interspecific call is the distress call [56, 63]. Distress calls are often convergent perhaps because the convergent form encourages interspecific mobbing of a predator [56]. There also is no apparent pressure for divergence in this signal; a potential prey item profits from interference from anyone, and interspecific mobbing is beneficial to all individuals who forage at the same site, as increased numbers of mobbers dilute predation risk, and the removal of a predator benefits all fellow prey items [63].

Benefits of more elaborate interspecific communication generally arise with the presence of a common predator or when resource levels are affected by the presence of a heterospecific [64, 65]. For example, cooperation, facilitated by communication, can reduce energy spent on predator avoidance or vigilance, as seen in the relationship between the dwarf mongoose (*Helogale parvula*) and hornbill (*Tockus flavirostris*) [64]. The presence of the alarm-calling

hornbill reduces the amount of time the dwarf mongoose spends watching for predators [64]. Cooperation, facilitated by communication, can also increase the foraging gains of one or both species, as seen in the relationship between the greater honeyguide (*Indicator indicator*) and humans (*Homo sapiens*) [65]. The greater honeyguide finds a bee hive food source and recruits humans, who break it open and provide easier access to the food items within [65]. Interspecific territoriality, advertised through signals, can also arise when a later arriving species invades a habitat previously held by another species, as seen in sympatric warblers [66]. Territoriality between species probably only occurs when niches do not diverge due to a simple habitat and/or limited resources [66]. Known interspecific 'contact' calls have varied functions, from mediating flock cohesion in some mixed-species bird flocks to possibly regulating space between groups of tamarins (*Saguinus fuscicollis* and *S. imperator*) [62]. Contact calls may encompass early forms of territorial and/or cooperative signals.

Research into interspecific communication is sparse. However, it is important to know the extent to which interspecific communication occurs, as knowledge in this field can influence interpretation of the adaptive significance of all types of communication.

Correlations vs. Interactions vs. Communication

Determining the existence of interspecific communication is a lengthy process. Communication is defined as the transfer of information from sender to receiver, through signals [51]. At least one individual in the sender-receiver dyad must benefit from this transfer of information [51]. Therefore, once a potential interspecific association and/or signal is found, it must be determined that the signals in question actually provide information, and that heterospecifics actually receive and use that information. One accepted indication of the receipt and use of information by a (heterospecific) receiver is the elicitation of a response or change in

receiver behavior following the signal [51]. However, it must be shown that the response is in fact elicited by the signal and is not due to chance or confounding variables.

Initially, correlations between potential interspecific signals and potential heterospecific responses can be sought. If a correlation is found, observational and experimental study of patterns surrounding signal production and potential heterospecific response should help indicate whether there are actual cause and effect interactions between the signal and response. For example, in order for interactions to be established, the signal should reliably precede the response, within a reasonable window of time, and the discovered correlation between signal and response should hold up under experimental conditions.

In order for communication to firmly be established, the information or message transferred between sender and receiver must also be determined. Information included in signals can be determined by comparison of signals across demographic groups [54, 57], correlation of signal production to various sender and receiver conditions [51], and observation of behavioral change in the receiver following the signal. Mathematical analysis of signal coding rules can aid in this endeavor [51].

The first step in the discovery of interspecific communication is the broad examination of heterospecific behaviors for possible correlations between species in those behaviors. Only after correlations have been found does it make sense to observationally, experimentally, and mathematically test for actual interspecific interactions and communication.

Peromyscus

Peromyscus is an excellent model for the study of interspecific communication because of its abundance [40], species diversity [40], and the overlap of ranges and required resources

between species [40, 46]. Ecological divergence between species appears to occur on the basis of microhabitat segregation [40]. However, the plasticity of microhabitat use within this genus [40], and the fact that both intra- and interspecific territoriality exists in some species at high densities (>25 mice per hectare) [40] suggest that interspecific interactions may be important to the overall ecology of *Peromyscus*.

P. californicus and *P. boylii* live syntopically in woodland habitat in the coastal California mountains [46]. There appears to be habitat partitioning, whereby a large number of *P. californicus* correlates to fewer *P. boylii* [46]. There is some food partitioning as well, probably due primarily to size differences between the species [46]. However, both species are active at the same time of night, and home ranges and core areas overlap extensively between species [46].

Ultrasonic vocalizations (USVs) are produced by *Peromyscus* in this shared habitat [1]. The function of these signals is unknown, though there are statistically significant differences in the average acoustic frequency of USVs between species (see Chapter II; Tables 1-5). A brief survey of correlations in the number of USVs produced by each species within a time interval should indicate whether interspecific interactions and/or communication mediated by USVs are plausible. A negative correlation between species in USV production could indicate temporal partitioning, and therefore an intraspecific function [55], or could possibly indicate that USVs are used to inhibit heterospecific activity, regulating space and/or resources between competing species. A positive correlation could indicate an interspecific function.

Objectives

The overall objective of this study was to determine whether there are correlations (which could indicate interspecific interactions) in the number of USVs produced by *P. californicus* with the number produced by *P. boylii*. To determine whether there is temporal or seasonal partitioning of USVs by species, a between-species comparison was made of: 1) the number of USVs produced by each species within each month, and 2) the number of USVs produced within each minute interval throughout the night (throughout the season). Additionally, to determine how close in time USVs from a heterospecific typically occur, the timing of USVs relative to USVs from a heterospecific mouse was examined. Time intervals investigated included: night (throughout the field season) and 30, 10, 5 and 1 minute intervals within a night.

Methods

Data Collection

For data collection, spectrographic analysis, terminology, and USV classification methods, see Chapter II. The same set of USV recordings and data as analyzed in Chapter II were used for this analysis.

Statistical Analysis

The entire set of USV data (All Vocalizations) was inspected for any trends in the overall timing of USV production by species. It was important to use All Vocalizations in order to have a sufficient number of USVs to analyze the timing of USV production. A frequency histogram of the number of vocalizations of each species per minute throughout the night (using all nights at once) was produced to look for trends in call production by time of night. Additionally, the number of vocalizations recorded per month was examined to look for monthly trends in call

production. Descriptive statistics were produced for the number of vocalizations per night by species during: 1) all nights when vocalizations by that species were recorded, 2) nights when both species called, and 3) nights when only one species called. T-tests and chi-square analysis were used for between-species comparisons over these same intervals.

To determine whether USVs were typically produced alone or in clusters (several USVs typically produced in a row, or within a short time interval from other USVs), the number of vocalizations throughout the field season produced within the same minute as any other USV was counted. The number of USVs produced within 5 minutes of any other USV was also counted. To look for patterns of heterospecific clustering of USVs, the number of vocalizations produced throughout the season within the same minute as an USV from a heterospecific mouse was counted. The number produced within 5 minutes of an USV from a heterospecific was also counted. The species which first vocalized during each cluster of vocalizations was counted, and the resulting count inspected to determine whether either species was the typical initiator of a heterospecific USV cluster.

Using All Vocalizations, a biplot of the number of vocalizations from *P. californicus* (x) versus the number of vocalizations from *P. boylii* (y) was created. A plot was made for the entire field season on nights when vocalizations were recorded. Plots were also made for 1, 10, and 30 minute intervals within each night where both species produced more than 3 vocalizations. The 10 and 30 minute intervals were used to detect larger behavioral trends, rather than likely interspecific interactions. A Pearson's correlation statistic was produced for each plot, to determine whether there was a significant relationship between the number of vocalizations of one species with that of the other within any of the time intervals. For all nights when both species produced more than 3 vocalizations and for the nights when significant within-minute

interspecific correlations were found, the distribution of motifs used by each species was compared to the overall distribution of motifs by species (in the All Vocalizations dataset) via chi-square analysis, to determine whether there was any likely relationship between motif and interspecific interaction. Correlation statistics were calculated in SPSS 16.0 and chi-square analyses was conducted using Microsoft Excel 2007.

Results

Peromyscus USVs were recorded on 77% of nights (95 of 123) when data collection equipment was set out. *Peromyscus californicus* were recorded on 69 nights, and *P. boylii* were recorded on 68 nights. On 34% of all nights (42 out of 123), both species vocalized. The 53 nights when only one species vocalized were divided nearly equally between *P. californicus* (27) and *P. boylii* (26).

Vocalizations of both species occurred throughout the night, from approximately 6:30 PM through 5:30 AM, with no apparent partitioning of timing in USV production between species (Fig. 4). Both species showed an overall peak in vocalization activity between approximately 8:00 and 10:00 PM (Fig. 4). *Peromyscus californicus* showed a peak in vocalization in February and *P. boylii* showed a peak in April (Fig. 5).

Both species produced a similar number of vocalizations per night, over all nights (*P. californicus* –average 7, median 2, SD 19.7, range 1-116; *P. boylii* – average 8, median 4, SD 26.5, range 1-218; n=137, t=.16, p=.87). T-tests showed no significant differences between species in the number of USVs produced on nights when both species called or on nights when each species called alone (together –n= 42, t=1.16, p=.25; alone – n=53, t=.81, p=.42; Table 7). On the 42 nights when both species vocalized, 62% percent of all *P. californicus* and 82% of all

P. boylii vocalizations were produced. A chi square test comparing the number of vocalizations on 'alone' vs. 'together' nights for each species showed that both species vocalized more than expected by chance on 'together' nights, or nights when both species vocalized ($\chi 2$= 54.63, df= 1, p<.01). The night of 3-April was an outlier, in that a total of 334 1-5 SV USVs were recorded (221 more USVs than recorded on any other night). Even with this outlier night removed, 51% of *P. californicus* vocalizations and 70% of *P. boylii* vocalizations were produced on nights when both vocalized. With 3-Apr removed from the data, chi-square analysis showed *P. boylii* still produced more USVs than expected by chance on nights when both species vocalized; however, *P. californicus* produced slightly more USVs than expected by chance on nights when only *P. californicus* vocalized ($\chi 2$= 28.97, df= 1, p<.01).

Peromyscus USVs showed a clustered pattern of distribution throughout a given night. Seventy-seven percent of all vocalizations (241) occurred within 5 minutes of any other 1-5 SV USV, heterospecific or conspecific. Forty-five percent of all 1-5 SV USVs (472 out of 1050) occurred within 5 minutes of an USV from a heterospecific, and 39% (408 out of 1050) occurred within 1 minute of an USV from a heterospecific (Table 8). However, with data from the outlier night 3-April removed (when all vocalizations occurred within 5 minutes of a heterospecific call), only 19% of vocalizations (138 out of 716) occurred within 5 minutes of a heterospecific, and only 13% (94 of 716) occurred within 1 minute of an USV from a heterospecific (Table 8).

Thirty-seven percent of *P. californicus* USVs and 52% of *P. boylii* USVs occurred within 5 minutes of heterospecific USVs (19% *P. californicus* and 20% *P. boylii* with 3-April removed; Table 8). Vocalizations were produced within 5 minutes of a heterospecific call on half of the nights when both species vocalized (21 out of 42 nights; Table 8). Both species initiated USV clusters a comparable number of times (5min: *P. californicus* – 14, *P. boylii* – 16; 1 min: *P.californicus* – 11, *P. boylii* – 11; 3-April removed from 1 min comparison because the

vocalizations during that night were nearly continuous, so that the first individual to vocalize during a minute was unlikely to be a true initiator).

Correlations were found between numbers of *P. californicus* vocalizations and numbers of *P. boylii* vocalizations, but not consistently throughout the data set. A positive correlation was shown between number of vocalizations by each species during each night over the entire field season ($R=.66$, $p<.01$), but when the outlier night of 3-April was removed, there was no correlation ($R=.04$, $p=.71$). Within the 11 nights when both species produced more than 3 vocalizations each, 8 nights showed a significant positive correlation between species vocalizations within 1 minute and 10 minute intervals (Table 9). Seven nights showed a significant positive correlation between species vocalizations within 30 minute intervals (Table 9).

On the 11 nights when both species produced more than 3 vocalizations each, and on the 8 nights when significant within-minute correlations in USV production were found between species, motif use by *P. californicus* was altered compared to the entire All Vocalizations dataset. When 3-April was removed from the data, *P. californicus* showed a significant difference in motif distribution as compared to the entire dataset on the nights when both species produced more than 3 vocalizations each ($\chi^2=12.41$, $df=4$, $p=.01$; Fig. 7), producing a larger proportion of 5-SV than in the All Vocalizations dataset. Similarly, when 3-April was removed from the data, *P. californicus* showed a significant difference in motif distribution as compared to the entire dataset on the nights when significant within-minute correlations in USV production between species were found ($\chi^2=19.66$, $df=4$, $p<.01$; Fig. 7), producing a larger proportion of 5-SV than in the All Vocalizations dataset. *P.boylii*, however, showed no significant difference from the overall data set in the proportion of each motif produced on either the nights when both species produced more than 3 vocalizations ($\chi^2= 3.85$, $df=4$, $p=.43$) or the nights when significant within-

minute interspecific correlations were found (χ^2=2.45, df=4, p=.65). On the outlier night 3-April, when mere inspection of a graph of vocalizations over time shows that both species were vocalizing at the same time (Fig. 6) and when all vocalizations occurred within 5 minutes of a heterospecific USV, *P. californicus* showed a significant difference in motif distribution (χ^2=34.57, df=4, p<.01; Fig. 7), however this time producing a larger proportion of 1-SV and a smaller proportion of 4- and 5-SV than in the All Vocalizations dataset. *P. boylii* motif distribution was unaffected (χ^2=6.96, df=4, p=.14).

Discussion

Both species produced a similar number of vocalizations over the field season. Each species vocalized on slightly more than half of all nights, at least during the breeding season. There was no suggestion of temporal partitioning during the night between species vocalizations. If temporal partitioning existed, it would suggest that USVs are intraspecific, likely with a mating function [55]. There may have been some seasonal partitioning, as each species shows a different peak in USVs over the field season. However, these data covered only one winter-spring season (5 monthly totals for each species), and additional data are needed to show a seasonal trend.

Though it was not uncommon for one USV to occur alone, they did tend to occur together. Both between and within nights there was a tendency for both species to vocalize at the same general time. In particular, a clear majority of *P. boylii* 1-5 SV USVs were produced on nights when both species vocalize (82%, or 70% with 3-April removed). However, it appears it was uncommon for either species to vocalize within 5 minutes of a heterospecific. Yet, on at least 8% of nights when USVs were recorded (8 out of 95 nights) this was not the case, and there was a high within-minute correlation in the vocalizing behavior of both species. Forty-seven percent of all 1-5 SV recorded (42% of all *P. californicus* and 53% of all *P. boylii*) were produced

on these 8 nights, suggesting that when one species vocalized much more than usual, the other species was highly likely to vocalize more than usual at the same time. However, neither species showed consistent behavioral changes (in motif, initiation of heterospecific USV clusters, or proportion of vocalizations produced in a heterospecific calling cluster) along with the within-minute heterospecific correlations to indicate interspecific versus intraspecific interactions.

The presence of significant behavioral changes (in motif, initiation of clusters, or proportion of vocalizations produced) would indicate that the behavior of one or both species could be changing in the presence of the other, and therefore that interactions could be occurring. For example, if motif distribution changed in the presence of heterospecific correlations in USV production, that could indicate that the different motifs may serve different functions and/or that certain motifs were more likely to be used for interspecific purposes. If the initiation of heterospecific clusters was different between species, it could indicate that interspecific interactions affected one species more than the other, as the species which alters its behavior is the species most affected [67]. If the proportion of vocalizations produced in the presence of USVs from a heterospecific was different between species, it could again indicate that interspecific interactions affected one species more than the other. The only behavioral changes found were a tendency for each species to vocalize more on nights when both species vocalized and a change in proportional motif use by *P. californicus*. When excluding the outlier 3-April, it appears that 5-SV may be used more commonly by *P. californicus* in the presence of heterospecifics (though 5-SV are still the least commonly used motif). However, on 3-April, the night when interspecific interactions seem most likely because all USVs occurred within 5 minutes of an USV from a heterospecific, the change in motif use by *P. californicus* was opposite from what was previously found. Therefore, no firm conclusions can be drawn about specific changes in motif use by *P. californicus* in the presence of heterospecifics. However, since the

motif changes are only seen in *P. californicus*, this suggests that any interspecific interactions found would probably affect *P. californicus* more than *P. boylii* [67].

Throughout the field season, 81% of 1-5 SVs occurred more than 5 minutes from an USV from a heterospecific (after the outlier night 3-April was removed). The remaining 19-20% of USVs of either species were produced within 5 minutes of a heterospecific USV. There was not a negative correlation found in USV production between species to indicate that calling by one species inhibits the other. In order to determine whether interspecific communication could be a factor in the USV function, further analysis is required. For example, randomizations could be done to determine whether heterospecific USVs occur together more often than would be expected by random chance [67]. Or, a Wilcoxon Signed-Rank test could be used [53] to compare the number of USVs each day which occurred either within or outside of a heterospecific USV cluster.

The fact that the majority of vocalizations from both species occurred on the same nights could also suggest that there may be other environmental variables or pressures that are important to USV production for both species. Further analysis of the data collected for this study could aid in determining if there are likely environmental variables affecting USV production in one or both species. Variables that may be of interest include moon phase, temperature, disruption of the focal area, emergence of young, density of mice, and presence of predators on or near the focal area. Only if environmental variables do not account for the heterospecific correlations in USV production should interspecific communication be pursued as a likely function of *Peromyscus* USVs.

The night of 3-April was an outlier in all respects. On this night, 331 of the 334 USVs were Classified Vocalizations, and it is therefore impossible to know how many individuals were

involved in USV production. However, an unusually high number of USVs were produced (334) compared to all other nights. Additionally, all USVs on 3-April occurred within 5 minutes of a USV from a heterospecific, and significant patterns of motif distribution and proportion of vocalizations produced by each species were different than other nights when both species produced more than 3 vocalizations each. There must be a reason for this. As interspecific interactions are often mediated by preadapted plastic behaviors [68, 69], it may be that, while none of the studied USV's primary function is interspecific communication, in rare situations, such as on 3-April, USVs are employed as a method to communicate during interspecific interactions. Or it may be that USVs are used to communicate within *Peromyscus* regardless of species, but only in rare situations, such as on 3-April, do heterospecifics interact. There were no obvious differences in weather, etc. on 3-April as compared to other nights during the field season. A further inspection of a variety of environmental variables on this night and other nights when significant correlations in heterospecific USVs were found may help clarify the apparently atypical behavior represented. Regardless, the existence of nights such as 3-April indicates that interspecific interactions between *P. californicus* and *P. boylii* are well worth investigation, whether or not the interactions are chiefly mediated by USVs.

CHAPTER IV

GENERAL CONCLUSIONS

Significant differences were found between species in the spectral characteristics of 1-5SV USVs, particularly in terms of frequency. Significant differences were also found between species in the proportional use of USV motifs, although both species use all five motifs, and 2-SV are the most commonly used motif in both species.

Both species had a similar median number of vocalizations per night, with no apparent temporal partitioning during the night, but with possible seasonal partitioning between species. USVs tend to occur in clusters, in which multiple USVs are produced within 5 minutes of at least one other USV. However, the majority of USVs occur more than 5 minutes from a USV produced by a heterospecific mouse.

Throughout the field season (with the removal of the outlier night) there was no correlation in the number USVs produced by each species. However, high positive correlations in USV production were found on some nights (such as 3-April). Further analysis of environmental data on these nights compared to other nights is necessary to determine whether the increase in USV production can be explained by environmental variables. If not, there may be interspecific interactions occurring which would be worth further investigation.

Peromyscus californicus shows an alteration from the overall data set in motif use in the presence of USVs produced by *P. boylii*. Therefore, *P. californicus* may be affected by the presence of *P. boylii* and/or there may be motifs which are more commonly used by *P. californicus* in the presence of a heterospecific mouse. These data did not show a consistent relationship between any motif and the presence of USVs produced by a heterospecific mouse, however. *Peromyscus boylii* did not show any alteration in motif distribution when in the presence of *P. californicus* USVs, as compared to the entire dataset.

The lack of negative correlations between species in the number of USVs produced indicates that 1-5SV USVs do not effectively inhibit activity by the other species. Therefore it is unlikely that these vocalizations function to preserve space or partition resources between species. The positive correlation suggests instead that these may be some type of assembly calls, possibly resource-recruitment (for foraging or vigilance benefits) or distress calls. Because the number of USVs recorded drops dramatically toward the end of the breeding season (end of May, June), it seems likely that these USVs have a function related in some way to reproduction. The species difference in within-species motif distribution may be related to the species difference in reproductive strategy.

It is worth noting that species classification of USVs by binary logistic regression and discriminant function analysis did not show 100% success, based on the USV assignments of the Overlay Vocalizations. Assignment of Classified Vocalizations to the incorrect species could have had an effect on the outcome of the clustering and correlation analysis. It is also possible that some of the USVs assigned to individuals via the overlay process were incorrectly assigned due to low resolution in the telemetry data (time recorded only to the minute, inconsistent signal detection, failed transmitters, large prediction interval for estimating distance by signal strength).

Therefore some caution should be used in the further study and use of these results. However, overall the species classification success of USVs was high, and between-species patterns found in the data were not suggestive of incorrect classification. It is therefore expected that the results are accurate. A chew-resistant method of attaching transmitters to resident mice, and more reliable transmitter batteries in future studies would aid in the collection of complete data regarding the location of the mice. Higher time resolution in the telemetry and video data would also significantly aid in precise USV assignment and accuracy of results.

Pseudoreplication may also have been a problem in this study, as the Classified Vocalizations were not identified to an individual, and therefore it is impossible to know how many individuals were represented in the dataset. Overlay Vocalizations (n = 393 USVs) were identified to 16 *P. californicus* and 23 *P. boylii* individuals. The data used for this study could be examined in a different way by averaging the spectral characteristics from all USVs identified to one individual and using only the individual averages in the between-species comparison (or, more concisely, by including species as a factor in a multivariate ANOVA). Preliminary analysis shows that species differences in acoustic frequency remain when species is included as a factor [M. Kalcounis-Rueppell, unpublished data].

Future Studies

Examination of USV production around different event types (such as predator, conspecific, heterospecific, or food presence, or emergence of young) could be helpful in determining the function of *Peromyscus* USVs [70]. Experimental studies where an individual of one species is introduced to an individual of the other species (or into its home range) may prove particularly helpful in clarifying the use of USVs as interspecific signals (and could help clarify changes in motif use by *P. californicus* in the presence of a heterospecific). It would also be

extremely beneficial to study the USV behavior of each of these species outside of the range of the other, to determine any character displacement effects of sympatry on USVs.

As more information is gathered regarding the context and function of USVs within *Peromyscus* and Muroidea, the known functions of USVs in related species can be compared with regard to genetic divergences (as determined from molecular phylogenies) and the ecological differences that may have been factors in the divergence. In this manner, the adaptive significance of USVs within *Peromyscus*, Muroidea, and Rodentia may eventually be understood.

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APPENDIX A. Tables and Figures.

Table 1. Descriptive Statistics for Measured Variables within 1 Syllable Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from the Overlay Vocalizations dataset used. Sample size refers to the number of vocalizations analyzed. Each syllable was measured for its duration, and minimum/maximum/peak(loudest) frequencies at the start, end, and point of maximum amplitude. Bandwidth is also measured at the start, end, and point of maximum amplitude within the syllable. Min Freq, Max Freq, and Total Bandwidth are measurements of the entire vocalization.

		P. californicus				***P. boylii***			
		n = 45				**n = 35**			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Duration	ms	30	330	110	10	30	310	170	10
Peak Freq(Start)	kHz	11.7	39.0	25.4	1.0	15.6	39.0	29.1	1.0
Min Freq(Start)	kHz	0.9	35.1	22.0	1.1	13.6	34.1	25.5	1.0
Max Freq(Start)	kHz	16.6	123.0	34.2	2.8	20.9	43.4	33.7	1.0
Bandw(Start)	kHz	5.8	122.0	12.2	3.0	6.3	12.6	8.2	0.3
Peak Freq(End)	kHz	13.6	36.1	23.7	0.9	15.6	36.1	27.4	0.8
Min Freq(End)	kHz	0.9	31.2	20.1	1.0	12.6	33.2	24.3	0.8
Max Freq(End)	kHz	20.5	123.0	31.0	2.2	20.9	41.5	32.8	0.8
Bandw(End)	kHz	5.8	122.0	10.8	2.4	5.3	16.6	8.5	0.3
Peak Freq(Max)	kHz	15.6	38.0	25.9	1.0	16.6	38.0	30.2	0.9
Min Freq(Max)	kHz	0.9	35.1	22.6	1.1	13.6	35.1	27.3	0.9
Max Freq(Max)	kHz	20.5	118.1	32.7	2.1	20.9	42.4	34.5	0.9
Bandw(Max)	kHz	6.8	117.1	10.2	2.3	6.3	10.7	7.2	0.1
Min Freq	kHz	0.9	31.2	19.7	1.0	12.6	33.2	23.3	0.8
Max Freq	kHz	22.4	123.0	35.2	2.8	20.9	43.4	35.2	0.9
Total Bandwidth	kHz	7.8	122.1	15.4	3.0	7.3	18.6	11.9	0.5

Table 2. Descriptive Statistics of Measured Variables within 2 Syllable Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from the Overlay Vocalizations dataset used. Sample size refers to the number of vocalizations analyzed. Each syllable was measured for its duration, and minimum/maximum/peak(loudest) frequencies at the start, end, and point of maximum amplitude. Bandwidth is also measured at the start, end, and point of maximum amplitude within the syllable. Interval is a measurement of the time between the end of a syllable and the start of the next syllable. Group Duration, Min Freq, Max Freq, and Total Bandwidth are measurements of the entire vocalization.

		P. californicus				***P. boylii***			
		n = 76				**n = 72**			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 1									
Duration	ms	40	380	150	10	40	350	160	10
Peak Freq(Start)	kHz	14.6	39.0	23.3	0.7	13.6	38.0	29.0	0.6
Min Freq(Start)	kHz	0.9	37.1	20.0	0.8	10.7	35.1	25.7	0.6
Max Freq(Start)	kHz	17.5	43.9	28.3	0.8	19.0	41.5	33.9	0.6
Bandw(Start)	kHz	2.9	39.0	8.3	0.4	5.8	12.6	8.2	0.2
Peak Freq(End)	kHz	14.6	34.1	21.4	0.6	15.6	35.1	26.4	0.5
Min Freq(End)	kHz	0.9	31.2	17.9	0.6	11.7	30.7	22.5	0.6
Max Freq(End)	kHz	19.5	39.0	26.6	0.6	20.0	41.9	31.5	0.6
Bandw(End)1	kHz	5.8	37.1	8.7	0.4	5.8	14.6	8.9	0.2
Peak Freq(Max)	kHz	14.6	40.0	23.5	0.8	15.6	39.0	29.6	0.6
Min Freq(Max)	kHz	11.7	37.1	20.6	0.8	13.6	36.1	26.8	0.6
Max Freq(Max)	kHz	19.5	44.9	28.3	0.8	20.9	43.4	34.1	0.6
Bandw(Max)	kHz	4.8	10.7	7.6	0.1	6.3	8.7	7.3	0.1
Interval1	ms	130	620	300	10	110	580	260	10
Syllable 2									
Duration	ms	10	310	130	10	40	280	140	10
Peak Freq(Start)	kHz	14.6	37.1	23.3	0.7	19.5	35.1	29.3	0.5
Min Freq(Start)	kHz	0.9	33.2	19.6	0.8	16.6	33.2	25.8	0.5
Max Freq(Start)	kHz	19.5	41.9	28.3	0.7	23.9	41.9	34.2	0.5
Bandw(Start)	kHz	6.8	38.0	8.7	0.4	5.8	13.6	8.4	0.2
Peak Freq(End)	kHz	14.6	38.0	23.3	0.8	16.6	35.1	29.3	0.4
Min Freq(End)	kHz	0.9	33.2	19.5	0.9	13.1	30.7	25.5	0.4
Max Freq(End)	kHz	20.5	123.0	30.0	1.4	21.9	41.0	34.5	0.5
Bandw(End)	kHz	5.8	122.0	10.5	1.5	5.3	15.6	8.9	0.2
Peak Freq(Max)	kHz	16.6	39.0	25.1	0.8	20.5	37.1	31.9	0.4
Min Freq(Max)	kHz	13.6	37.1	22.2	0.8	17.5	34.1	29.1	0.4
Max Freq(Max)	kHz	21.4	44.9	29.9	0.8	25.8	43.4	36.7	0.4
Bandw(Max)	kHz	6.8	9.7	7.7	0.1	6.3	25.8	7.5	0.3
Entire Vocalization									
Gr Duration	ms	180	880	430	20	250	720	400	10
Min Freq	kHz	0.9	31.2	16.786	0.673	10.7	29.7	21.675	0.514
Max Freq	kHz	22.4	123	32.08	1.439	25.8	43.4	37.379	0.374
Total Bandwidth	kHz	9.8	122.1	15.295	1.505	8.8	29.8	15.704	0.443

Table 3. Descriptive Statistics of Measured Variables within 3 Syllable Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from the Overlay Vocalizations dataset used. Sample size refers to the number of vocalizations analyzed. Each syllable was measured for its duration, and minimum/maximum/peak(loudest) frequencies at the start, end, and point of maximum amplitude. Bandwidth is also measured at the start, end, and point of maximum amplitude within the syllable. Interval is a measurement of the time between the end of a syllable and the start of the next syllable. Group Duration, Min Freq, Max Freq, and Total Bandwidth are measurements of the entire vocalization.

		P. californicus **n = 77**				***P. boylii*** **n = 40**			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 1									
Duration	ms	10	340	110	10	30	310	130	10
Peak Freq(Start)	kHz	15.6	36.1	21.2	0.5	12.6	34.1	25.6	0.9
Min Freq(Start)	kHz	12.6	33.2	18.0	0.5	7.8	32.2	22.2	1.0
Max Freq(Start)	kHz	20.5	41.0	26.0	0.5	20.0	39.5	30.6	0.9
Bandw(Start)	kHz	5.8	14.6	7.9	0.1	6.3	13.6	8.3	0.3
Peak Freq(End)	kHz	13.6	34.1	20.0	0.4	12.6	32.2	24.4	0.9
Min Freq(End)	kHz	10.7	31.2	16.8	0.4	8.7	29.2	20.5	0.8
Max Freq(End)	kHz	19.5	40.0	25.2	0.4	18.5	38.0	29.4	0.9
Bandw(End)	kHz	4.8	13.6	8.4	0.2	3.9	13.6	8.8	0.3
Peak Freq(Max)	kHz	16.6	36.1	20.9	0.4	13.6	36.1	26.7	0.9
Min Freq(Max)	kHz	13.6	33.2	18.0	0.4	10.7	33.2	23.9	0.9
Max Freq(Max)	kHz	21.4	41.0	25.9	0.4	18.0	40.5	31.2	0.9
Bandw(Max)	kHz	6.8	11.7	7.9	0.1	6.3	8.7	7.3	0.1
Interval	ms	120	710	230	10	110	550	220	110
Syllable 2									
Duration	ms	70	300	150	10	30	310	160	10
Peak Freq(Start)	kHz	14.6	37.1	21.4	0.6	12.6	35.1	27.5	0.8
Min Freq(Start)	kHz	0.9	32.2	18.0	0.6	10.2	31.2	24.4	0.8
Max Freq(Start)	kHz	19.5	116.2	27.5	1.3	17.5	41.0	33.1	0.8
Bandw(Start)	kHz	5.8	115.2	9.5	1.4	6.3	14.6	8.7	0.3
Peak Freq(End)	kHz	16.6	35.1	21.7	0.5	17.5	34.1	29.0	0.7
Min Freq(End)	kHz	12.6	31.2	18.4	0.5	16.1	32.2	25.7	0.6
Max Freq(End)	kHz	22.4	40.0	26.9	0.5	22.9	41.0	34.3	0.7
Bandw(End)	kHz	2.9	13.6	8.5	0.2	6.3	13.6	8.6	0.3
Peak Freq(Max)	kHz	17.5	36.1	23.2	0.5	19.5	36.1	31.6	0.6
Min Freq(Max)	kHz	14.6	33.2	20.4	0.5	17.5	33.2	28.9	0.6
Max Freq(Max)	kHz	21.4	41.0	28.0	0.5	23.9	41.0	36.2	0.6
Bandw(Max)	kHz	6.8	9.7	7.6	0.1	6.3	8.3	7.3	0.1
Interval	ms	140	530	270	10	100	450	260	10

Table 3, continued. Descriptive Statistics of Measured Variables within 3 Syllable Vocalizations.

		P. californicus n = 77				*P. boylii* n = 40			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 3									
Duration	ms	20	210	90	0.0	20	210	110	10
Peak Freq(Start)	kHz	12.6	39.0	21.2	0.7	13.6	36.1	28.2	0.8
Min Freq(Start)	kHz	7.8	35.1	18.1	0.7	11.2	33.2	24.7	0.8
Max Freq(Start)	kHz	18.5	44.9	26.2	0.7	20.0	40.5	33.5	0.8
Bandw(Start)	kHz	4.8	12.6	8.1	0.1	6.3	13.6	8.8	0.3
Peak Freq(End)	kHz	14.6	36.1	21.8	0.6	18.5	35.1	30.1	0.6
Min Freq(End)	kHz	0.9	33.2	18.1	0.6	15.6	32.2	26.4	0.6
Max Freq(End)	kHz	21.4	123.0	29.3	1.7	23.9	41.0	35.6	0.7
Bandw(End)	kHz	5.8	122.0	11.1	1.9	5.3	14.6	9.1	0.3
Peak Freq(Max)	kHz	15.6	38.0	23.2	0.6	20.5	36.1	31.8	0.6
Min Freq(Max)	kHz	12.6	35.1	20.3	0.6	15.6	33.2	28.9	0.6
Max Freq(Max)	kHz	20.5	42.9	28.0	0.6	26.3	41.0	36.3	0.6
Bandw(Max)	kHz	6.8	9.7	7.7	0.1	6.3	10.7	7.4	0.1
Entire Vocalization									
Gr Duration	ms	330	1190	590	20	390	1180	590	30
Min Freq	kHz	0.9	26.3	14.3	0.5	7.8	27.8	19.0	0.9
Max Freq	kHz	24.4	123.0	32.3	2.0	26.8	41.0	37.4	0.6
Total Bandwidth	kHz	9.7	122.1	18.0	2.2	11.3	27.3	18.4	0.8

Table 4. Descriptive Statistics of Measured Variables within 4 Syllable Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from the Overlay Vocalizations dataset used. Sample size refers to the number of vocalizations analyzed. Each syllable was measured for its duration, and minimum/maximum/peak(loudest) frequencies at the start, end, and point of maximum amplitude. Bandwidth is also measured at the start, end, and point of maximum amplitude within the syllable. Interval is a measurement of the time between the end of a syllable and the start of the next syllable. Group Duration, Min Freq, Max Freq, and Total Bandwidth are measurements of the entire vocalization.

		P. californicus **n = 28**				***P. boylii*** **n = 12**			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 1									
Duration	ms	20	330	90	10	60	290	130	20
Peak Freq(Start)	kHz	15.6	29.2	20.4	0.6	20.5	38.0	28.0	1.4
Min Freq(Start)	kHz	12.6	26.3	17.5	0.7	17.5	35.1	24.5	1.5
Max Freq(Start)	kHz	21.4	34.1	25.3	0.6	25.3	41.5	32.4	1.4
Bandw(Start)	kHz	6.8	9.7	7.8	0.1	5.8	10.7	7.8	0.4
Peak Freq(End)	kHz	16.6	24.4	19.0	0.4	17.5	33.2	24.8	1.3
Min Freq(End)	kHz	13.6	21.4	15.9	0.4	13.6	31.2	21.1	1.5
Max Freq(End)	kHz	21.4	29.2	24.1	0.4	24.4	40.0	30.5	1.2
Bandw(End)	kHz	5.8	11.7	8.2	0.3	7.3	14.6	9.3	0.6
Peak Freq(Max)	kHz	16.6	29.2	20.1	0.5	21.4	37.1	27.9	1.4
Min Freq(Max)	kHz	13.6	26.3	17.4	0.5	18.5	35.1	25.2	1.4
Max Freq(Max)	kHz	21.4	34.1	24.9	0.6	25.3	41.5	32.5	1.4
Bandw(Max)	kHz	5.8	8.7	7.5	0.1	6.3	7.8	7.3	0.2
Interval	ms	0.11	0.58	0.21	0.02	0.13	0.41	0.22	0.02
Syllable 2									
Duration	ms	80	270	140	10	90	160	140	10
Peak Freq(Start)	kHz	14.6	31.2	20.2	0.9	22.4	32.2	28.0	0.9
Min Freq(Start)	kHz	11.7	28.3	17.2	0.9	18.5	29.2	24.8	1.1
Max Freq(Start)	kHz	19.5	35.1	25.1	0.9	27.3	37.5	33.5	0.8
Bandw(Start)	kHz	5.8	10.7	7.8	0.2	6.3	13.6	8.7	0.6
Peak Freq(End)	kHz	17.5	31.2	21.3	0.8	23.4	37.5	29.3	1.2
Min Freq(End)	kHz	14.6	28.3	18.0	0.8	20.5	30.7	25.0	1.1
Max Freq(End)	kHz	22.4	36.1	26.7	0.7	29.2	41.5	34.8	1.1
Bandw(End)	kHz	5.8	11.7	8.6	0.3	6.8	12.6	9.7	0.6
Peak Freq(Max)	kHz	18.5	35.1	22.6	1.0	24.4	37.1	31.5	1.0
Min Freq(Max)	kHz	14.6	32.2	19.7	1.0	22.4	35.1	28.8	1.0
Max Freq(Max)	kHz	23.4	40.0	27.5	0.9	29.2	41.5	36.2	1.0
Bandw(Max)	kHz	6.8	9.7	7.8	0.1	6.3	7.8	7.4	0.2
Interval	ms	170	430	240	10	190	260	230	10
Syllable 3									
Duration	ms	90	220	130	10	90	160	130	10
Peak Freq(Start)	kHz	9.7	32.2	19.4	1.0	23.4	32.2	28.7	1.1
Min Freq(Start)	kHz	6.8	29.2	16.1	1.0	17.5	29.2	25.0	1.2
Max Freq(Start)	kHz	13.6	37.1	24.5	1.0	29.2	38.0	34.8	0.9
Bandw(Start)	kHz	6.8	11.7	8.4	0.2	6.8	15.6	9.7	0.8
Peak Freq(End)	kHz	16.6	33.2	21.2	0.8	25.3	35.1	29.3	1.0
Min Freq(End)	kHz	14.6	30.2	18.0	0.8	19.5	33.2	24.9	1.2
Max Freq(End)	kHz	19.5	38.0	26.3	0.8	31.2	40.0	35.4	1.1

Table 4, continued. Descriptive Statistics of Measured Variables within 4 Syllable Vocalizations.

		P. californicus n = 28				*P. boylii* n = 12			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 3 cont'd									
Bandw(End)	kHz	4.8	11.7	8.3	0.3	6.3	12.6	10.5	0.6
Peak Freq(Max)	kHz	16.6	35.1	22.7	1.0	25.3	37.1	31.9	1.0
Min Freq(Max)	kHz	13.6	32.2	19.7	1.0	21.4	34.1	28.9	1.1
Max Freq(Max)	kHz	21.4	40.0	27.3	1.0	30.2	41.5	36.5	1.0
Bandw(Max)	kHz	6.8	8.7	7.6	0.1	6.8	8.7	7.6	0.2
Interval	ms	190	420	240	10	180	280	220	10
Syllable 4									
Duration	ms	20	140	70	10	80	140	100	10
Peak Freq(Start)	kHz	10.7	32.2	19.4	1.1	21.4	34.1	29.8	0.9
Min Freq(Start)	kHz	7.8	29.2	16.5	1.0	19.5	30.2	26.1	0.9
Max Freq(Start)	kHz	17.5	37.1	24.8	1.1	30.2	39.0	35.6	0.7
Bandw(Start)	kHz	6.8	11.7	8.2	0.2	6.3	13.6	9.5	0.7
Peak Freq(End)	kHz	16.6	33.2	21.5	0.9	24.4	33.2	29.9	1.0
Min Freq(End)	kHz	13.6	30.2	18.1	0.9	20.5	30.2	25.9	1.0
Max Freq(End)	kHz	21.4	38.0	26.4	0.9	32.2	41.0	36.1	0.8
Bandw(End)	kHz	6.8	11.7	8.2	0.2	6.8	14.6	10.1	0.7
Peak Freq(Max)	kHz	16.6	35.1	21.9	1.0	25.3	35.1	31.5	1.0
Min Freq(Max)	kHz	13.6	32.2	19.0	1.0	22.4	32.2	28.7	0.9
Max Freq(Max)	kHz	22.4	40.0	26.7	1.0	30.2	39.5	36.3	0.9
Bandw(Max)	kHz	6.8	9.7	7.7	0.1	6.8	8.3	7.6	0.1
Entire Vocalization									
Gr Duration	ms	570	1520	760	40	590	1040	760	30
Min Freq	kHz	6.8	21.4	13.5	0.6	13.6	27.3	19.8	1.1
Max Freq	kHz	24.4	40.0	28.4	0.9	34.1	41.5	38.5	0.7
Total Bandwidth	kHz	9.8	24.4	14.9	0.7	14.1	24.4	18.7	0.9

Table 5. Descriptive Statistics of Measured Variables within 5 Syllable Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from the Overlay Vocalizations dataset used. Sample size refers to the number of vocalizations analyzed. Each syllable was measured for its duration, and minimum/maximum/peak(loudest) frequencies at the start, end, and point of maximum amplitude. Bandwidth is also measured at the start, end, and point of maximum amplitude within the syllable. Interval is a measurement of the time between the end of a syllable and the start of the next syllable. Group Duration, Min Freq, Max Freq, and Total Bandwidth are measurements of the entire vocalization.

		P. californicus **n = 6**				***P. boylii*** **n = 2**			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 1									
Duration	ms	20	110	40	20	60	120	90	30
Peak Freq(Start)	kHz	18.5	21.4	20.5	0.5	22.4	31.2	26.8	4.4
Min Freq(Start)	kHz	15.6	18.5	17.5	0.5	20.5	28.3	24.4	3.9
Max Freq(Start)	kHz	23.4	25.3	24.9	0.4	26.8	35.6	31.2	4.4
Bandw(Start)	kHz	6.8	7.8	7.4	0.2	6.3	7.3	6.8	0.5
Peak Freq(End)	kHz	17.5	20.5	19.1	0.5	27.3	28.3	27.8	0.5
Min Freq(End)	kHz	13.6	15.6	14.6	0.3	24.9	25.3	25.1	0.2
Max Freq(End)	kHz	23.4	25.3	24.6	0.4	32.7	33.6	33.2	0.5
Bandw(End)	kHz	7.8	11.7	9.9	0.6	7.3	8.7	8.0	0.7
Peak Freq(Max)	kHz	18.5	20.5	19.5	0.3	33.2	33.2	33.2	0.0
Min Freq(Max)	kHz	15.6	17.5	16.8	0.4	30.2	30.2	30.2	0.0
Max Freq(Max)	kHz	23.4	25.3	24.4	0.3	36.6	37.5	37.1	0.5
Bandw(Max)	kHz	6.8	7.8	7.6	0.2	6.3	7.3	6.8	0.5
Interval	ms	130	230	160	20	210	210	210	0.0
Syllable 2									
Duration	ms	110	140	130	110	40	90	60	30
Peak Freq(Start)	kHz	15.6	21.4	18.3	1.3	26.3	33.2	29.8	3.5
Min Freq(Start)	kHz	13.6	17.5	15.8	0.8	23.4	29.2	26.3	2.9
Max Freq(Start)	kHz	20.5	27.3	23.4	1.4	31.7	37.5	34.6	2.9
Bandw(Start)	kHz	5.8	9.7	7.6	0.7	8.3	8.3	8.3	0.0
Peak Freq(End)	kHz	17.5	19.5	18.7	0.4	29.2	30.2	29.7	0.5
Min Freq(End)	kHz	14.6	15.6	15.2	0.2	25.3	27.3	26.3	1.0
Max Freq(End)	kHz	23.4	24.4	24.2	0.2	34.6	34.6	34.6	0.0
Bandw(End)	kHz	7.8	9.7	8.9	0.4	7.3	9.2	8.3	1.0
Peak Freq(Max)	kHz	18.5	21.4	19.3	0.6	32.2	33.2	32.7	0.5
Min Freq(Max)	kHz	15.6	18.5	16.4	0.6	29.2	30.2	29.7	0.5
Max Freq(Max)	kHz	23.4	26.3	24.9	0.5	36.6	37.5	37.1	0.5
Bandw(Max)	kHz	7.8	9.7	8.6	0.5	7.3	7.3	7.3	0.0
Interval	ms	230	290	250	10	130	170	150	20
Syllable 3									
Duration	ms	110	140	130	10	130	140	140	0.0
Peak Freq(Start)	kHz	16.6	21.4	19.1	1.1	29.2	33.2	31.2	2.0
Min Freq(Start)	kHz	13.6	19.5	16.4	1.0	27.3	30.2	28.8	1.5
Max Freq(Start)	kHz	21.4	26.3	23.8	1.0	35.6	36.6	36.1	0.5
Bandw(Start)	kHz	6.8	7.8	7.4	0.2	6.3	8.3	7.3	1.0
Peak Freq(End)	kHz	17.5	22.4	19.5	0.8	32.2	33.2	32.7	0.5
Min Freq(End)	kHz	15.6	19.5	16.8	0.7	29.2	30.2	29.7	0.5
Max Freq(End)	kHz	22.4	27.3	24.8	0.8	35.6	36.6	36.1	0.5

Table 5, continued. Descriptive Statistics of Measured Variables within 5 Syllable Vocalizations.

		P. californicus n = 6				*P. boylii* n = 2			
Variable	**units**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Mean**	**Std. Error**
Syllable 3 cont'd									
Bandw(End)	kHz	6.8	9.7	8.0	0.5	6.3	6.3	6.3	0.0
Peak Freq(Max)	kHz	19.5	22.4	21.4	0.5	33.2	34.1	33.7	0.5
Min Freq(Max)	kHz	16.6	19.5	18.5	0.5	31.2	32.2	31.7	0.5
Max Freq(Max)	kHz	24.4	27.3	26.3	0.5	37.5	39.5	38.5	1.0
Bandw(Max)	kHz	7.8	7.8	7.8	0.0	6.3	7.3	6.8	0.5
Interval	ms	210	260	240	10	220	230	220	0.0
Syllable 4									
Duration4	ms	90	140	110	10	120	120	120	0.0
Peak Freq(Start)	kHz	15.6	21.4	17.7	1.0	29.2	32.2	30.7	1.5
Min Freq(Start)	kHz	11.7	16.6	14.2	0.8	26.3	28.3	27.3	1.0
Max Freq(Start)	kHz	20.5	26.3	22.4	1.0	34.6	35.6	35.1	0.5
Bandw(Start)	kHz	6.8	9.7	8.2	0.5	7.3	8.3	7.8	0.5
Peak Freq(End)	kHz	18.5	21.4	19.7	0.6	31.2	34.1	32.7	1.5
Min Freq(End)	kHz	14.6	17.5	15.8	0.5	28.3	31.2	29.8	1.5
Max Freq(End)	kHz	22.4	26.3	24.5	0.7	35.6	38.5	37.1	1.5
Bandw(End)	kHz	6.8	10.7	8.7	0.7	7.3	7.3	7.3	0.0
Peak Freq(Max)	kHz	16.6	22.4	19.9	0.9	33.2	34.1	33.7	0.5
Min Freq(Max)	kHz	13.6	19.5	16.9	1.0	31.2	31.2	31.2	0.0
Max Freq(Max)	kHz	21.4	27.3	24.7	1.0	38.5	38.5	38.5	0.0
Bandw(Max)	kHz	7.8	7.8	7.8	0.0	7.3	7.3	7.3	0.0
Interval	ms	210	260	230	10	210	230	220	10
Syllable 5									
Duration	ms	20	130	60	20	70	80	80	0.0
Peak Freq(Start)	kHz	15.6	16.6	16.0	0.2	24.4	33.2	28.8	4.4
Min Freq(Start)	kHz	11.7	13.6	12.4	0.4	21.4	30.2	25.8	4.4
Max Freq(Start)	kHz	19.5	21.4	20.7	0.4	28.8	38.5	33.7	4.9
Bandw(Start)	kHz	7.8	9.7	8.2	0.4	7.3	8.3	7.8	0.5
Peak Freq(End)	kHz	17.5	20.5	18.3	0.6	31.2	33.2	32.2	1.0
Min Freq(End)	kHz	14.6	16.6	15.4	0.4	29.7	30.2	30.0	0.3
Max Freq(End)	kHz	22.4	25.3	23.6	0.6	35.6	37.5	36.6	1.0
Bandw(End)	kHz	6.8	9.7	8.2	0.5	5.8	7.3	6.6	0.8
Peak Freq(Max)	kHz	16.6	19.5	17.7	0.6	33.2	33.2	33.2	0.0
Min Freq(Max)	kHz	13.6	16.6	14.8	0.6	30.2	30.2	30.2	0.0
Max Freq(Max)	kHz	21.4	24.4	22.4	0.5	37.5	37.5	37.5	0.0
Bandw(Max)	kHz	6.8	7.8	7.6	0.2	7.3	7.3	7.3	0.0
Entire Vocalization									
Gr Duration	ms	820	1060	940	50	860	890	880	10
Min Freq	kHz	11.7	14.6	13.8	0.6	20.5	24.9	22.7	2.2
Max Freq	kHz	26.3	27.3	26.7	0.2	38.5	39.5	39.0	0.5
Total Bandwidth	kHz	11.7	14.6	12.9	0.5	13.6	19.0	16.3	2.7

Table 6. Species Predictors used for Classification of Classified Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from the Overlay Vocalizations dataset used for variable selection, and to determine the classification success using the chosen variables. Sample size refers to the total number of vocalizations in the All Vocalizations dataset (n = 1050 USVs) classified using the Logistic Regression (LR) and Discriminant Function Analysis (DFA) models. Duration refers to the length of a syllable; MinFreqMax refers to the minimum frequency at the point of maximum amplitude within a syllable; BandwMax refers to the bandwidth at the point of maximum amplitude within a syllable; MaxFreqEnd refers to the maximum frequency at the end of a syllable. The number at the end of each variable refers to the syllable in reference. Due to the small sample size of 5-SV, the R^2 was again 1. However, as the model was significant and the 5-SV were classified about equally between species, with a large difference between species in the mean frequency, the classifications were accepted as valid.

			Binary Logistic Regression						Discriminant Function Analysis						
Motif	**total n**	**Variables included in Model**	**LR Model χ^2**	**df**	**p**	**R^2**	**Correct Classification of *P. boylii* Overlay Vocalizations**	**Correct Classification of *P. californicus* Overlay Vocalizations**	**DFA Model χ^2 statistic**	**df**	**p**	**Correct Classification of *P. boylii* Overlay Vocalizations**	**Correct Classification of *P. californicus* Overlay Vocalizations**	**Agreement Between LR and DFA Assignments**	**Final Success Classifying Overlay Vocalizations**
1-SV	264	Duration MinFreqMax	25.07	2	< .01	0.35	66%	81%	24.08	2	< .01	69%	70%	92%	74%
2-SV	393	MinFreqMax2	48.63	1	< .01	0.37	89%	67%	51.17	1	< .01	89%	67%	100%	78%
3-SV	267	MinFreqMax2 BandwMax1	76.3	2	< .01	0.66	76%	90%	78.3	2	< .01	88%	83%	86%	86%
4-SV	97	MaxFreqEnd4 BandwEnd3	30.74	2	< .01	0.76	75%	89%	32.65	2	< .01	92%	82%	78%	85%
5-SV	29	MinFreqMax1	9	1	0.003	1	100%	100%	21.81	1	< .01	100%	100%	100%	100%

Table 7. Vocalizing Behavior by Species, Comparing Nights When Both Species Vocalized to Nights When Only One Species Vocalized.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008 using All Vocalizations dataset (n = 1050 USVs). "Together" refers to nights when both species produced USVs. "Alone" refers to nights when only the referenced species produced USVs. The p-value refers to a between-species comparison of the numbers of USVs produced. These data include the outlier night of 3-April, when 334 USVs were produced (218 by *P. boylii* and 116 by *P. californicus*).

		Total USVs	Total Nights	Average per Night	Median per Night	p
Together	***P. californicus***	314	42	7	2	.25
	P. boylii	447	42	11	4	
Alone	***P. californicus***	193	27	7	2	.42
	P. boylii	96	26	4	2	

Table 8. Heterospecific Clusters of USVs.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from All Vocalizations dataset (n = 1050 USVs) used. Without the outlier night 3-April (see text), n=716. Counts show the number of USVs from each species produced within 1-5 minutes of a heterospecific USV, and the number of nights when USVs were recorded within 1-5 minutes of a heterospecific, throughout the entire field season. Both species vocalized on 42 nights during the field season.

		# Nights when USVs produced	USVs within 1 Minute of Heterospecific (17 nights)	USVs within 5 Minutes of Heterospecific (21 nights)
All Nights	***P. californicus***	69	168	189
	P. boylii	68	240	283
Without 3-April	***P. californicus***	68	52	73
	P. boylii	67	42	65

Table 9. Correlation in Number of 1-5 SV USVs Produced by Each Species within 1, 10, and 30 Minute Intervals During Nights When Each Species Produced More Than 3 USVs.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008. Sample size refers to the number of USVs recorded on each night, broken down by species. All Vocalizations dataset used to find correlations between the number of USVs produced by each species on nights when each species produced more than 3 USVs.

		8-Feb	10-Feb	11-Feb	3-Mar	12-Mar	31-Mar	1-Apr	3-Apr	11-Apr	24-Apr	25-Apr
n	***P. californicus***	48	6	7	10	11	5	5	116	5	12	11
	P. boylii	15	7	19	6	5	14	14	218	6	4	6
1 minute intervals	**Pearson's Correlation Statistic**	**0.11**	**0.00**	**0.91**	**0.11**	**0.27**	**0.32**	**-0.01**	**0.80**	**0.37**	**-0.01**	**0.32**
	p-value	**< .01**	**0.91**	**< .01**	**< .01**	**< .01**	**< .01**	**0.82**	**< .01**	**< .01**	**0.83**	**< .01**
	sig	*****		*****	*****	*****	*****		*****	*****		*****
10 minute intervals	**Pearson's Correlation Statistic**	**0.32**	**0.61**	**0.91**	**0.42**	**0.34**	**0.55**	**0.00**	**0.87**	**0.68**	**-0.07**	**0.19**
	p-value	**< .01**	**< .01**	**< .01**	**< .01**	**< .01**	**< .01**	**1.00**	**< .01**	**< .01**	**.55**	**.11**
	sig	*****	*****	*****	*****	*****	*****		*****	*****		
30 minute intervals	**Pearson's Correlation Statistic**	**0.54**	**0.78**	**0.91**	**0.33**	**0.32**	**0.65**	**0.07**	**0.96**	**0.61**	**-0.19**	**0.56**
	p-value	**0.01**	**< .01**	**< .01**	**0.11**	**0.12**	**< .01**	**0.74**	**< .01**	**< .01**	**0.36**	**< .01**
	sig	*****	*****	*****			*****		*****	*****		*****

Figure 1. Spectrogram of 3-SV USVs from *Peromyscus californicus* and *P. boylii*.

Vocalizations recorded from free-living *P. californicus* and *P. boylii* in California in 2008. Representative 3-SV USVs recorded from (A) *P. californicus* and (B) *P. boylii*. Syllable and duration measurements indicated in (A).

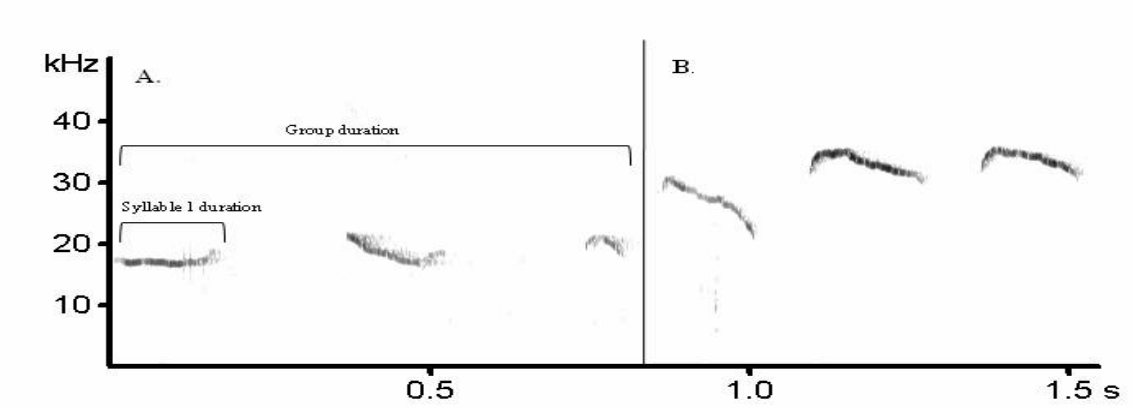


Figure 2. Sample Diagram of a Focal Area with Microphone Array.

Overhead view of a focal area on the study site in California, as seen from thermal imaging camera lens suspended ~30 ft above the ground. The numbered blue dots represent microphones. A diagram such as this was used as an aid in localizing an USV within the focal area, based on a comparison of the arrival time of the USV at different microphones within the microphone array.

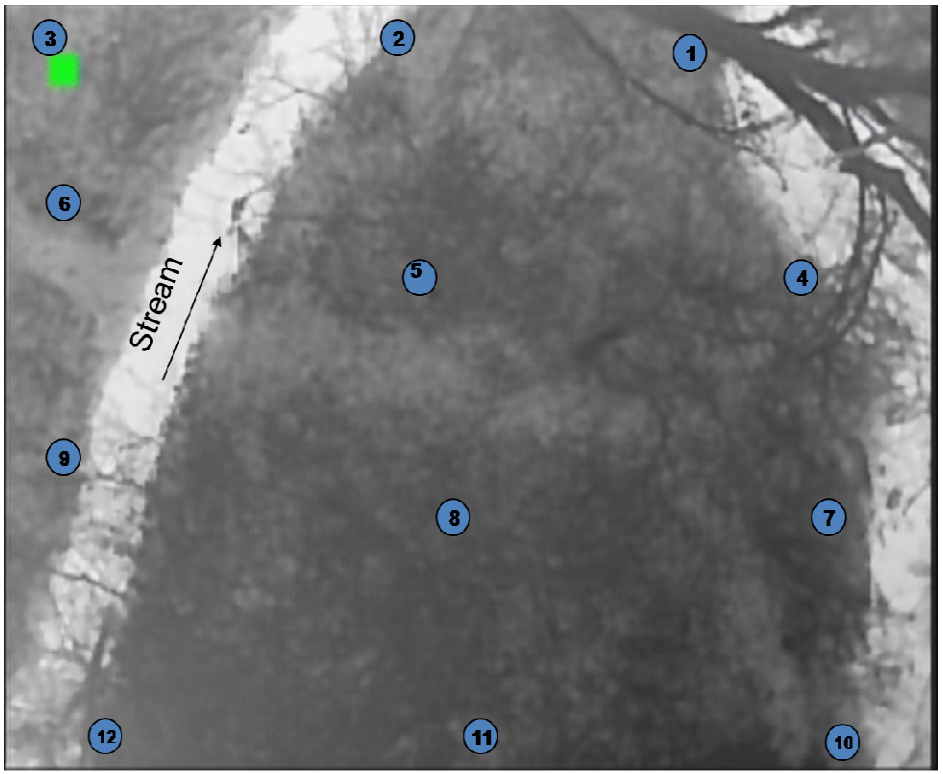
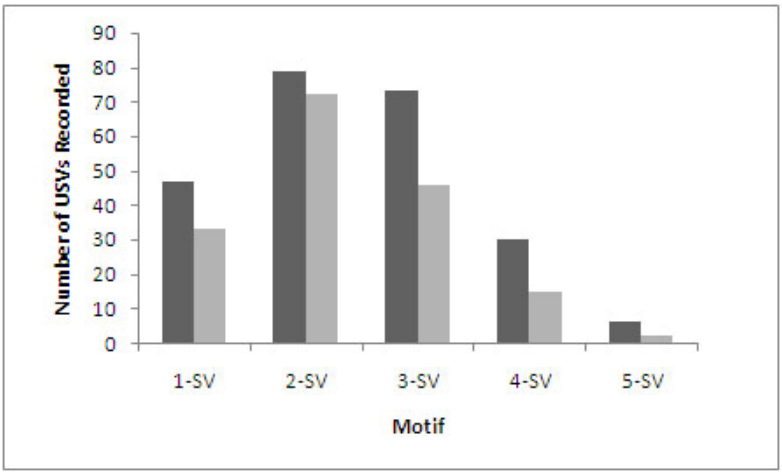


Figure 3. Motif Distribution by Species within Overlay Vocalizations and All Vocalizations.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008. Number of recorded USVs is broken down by species and motif to show the motif distribution by species. (A) Overlay Vocalizations (n = 393 USVs) are those that were assigned to individual mice using the overlay process. (B) All Vocalizations (n = 1050 USVs) encompasses both the Overlay Vocalizations and Classified Vocalizations datasets. *P. californicus* is represented by dark gray, and *P. boylii* by light gray.

A.



B.

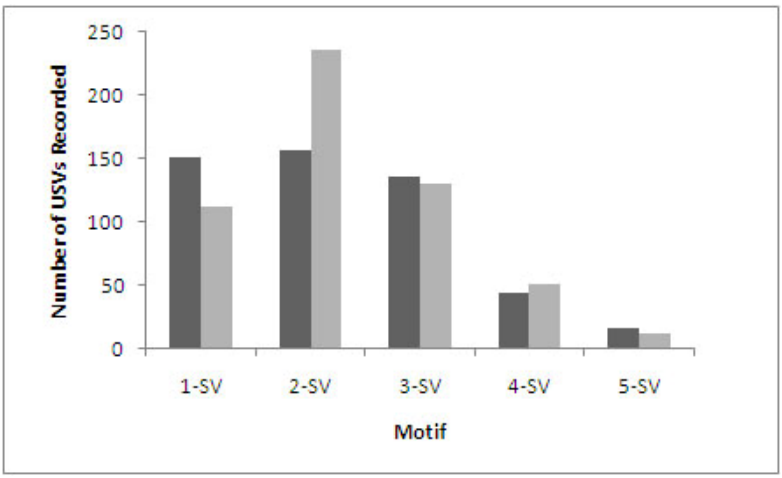


Figure 4. Frequency Histogram of Vocalizations by Time of Night throughout Field Season.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008; data from Overlay Vocalizations (n = 393 USVs) used. *P. californicus* is represented by dark gray, and *P. boylii* by light gray. Using data from all nights throughout the field season at once, USV counts per minute throughout the night were graphed to determine whether USVs were commonly produced at a particular time of night. For purposes of best illustration, the histogram below shows the data in 30 minute intervals.

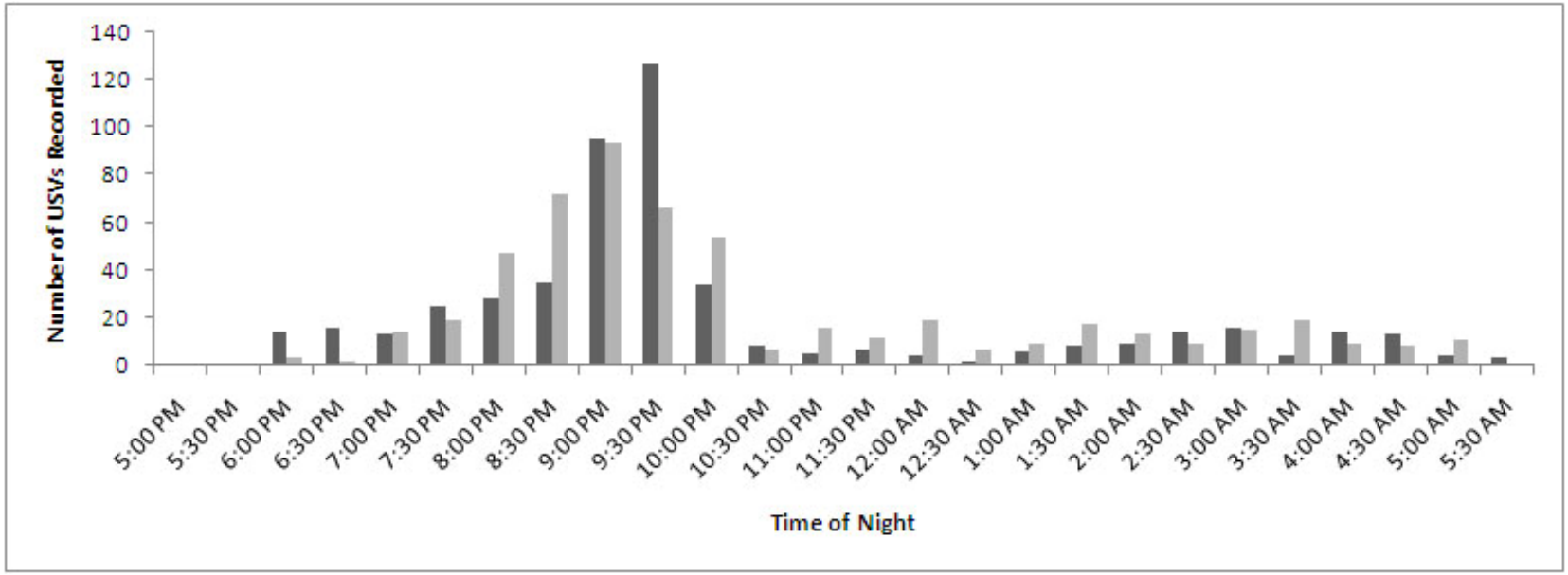


Figure 5. Number of USVs Produced per Month, by Species.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008. (A) Number of recorded USVs in All Vocalizations dataset is broken down by species and month to show the monthly distribution of USVs by species. (B) Monthly distribution of USVs by species without the outlier night 3-April, when 334 1-5 SV USVs were produced (32% of All Vocalizations). *P. californicus* is represented by dark gray, and *P. boylii* is represented by light gray.

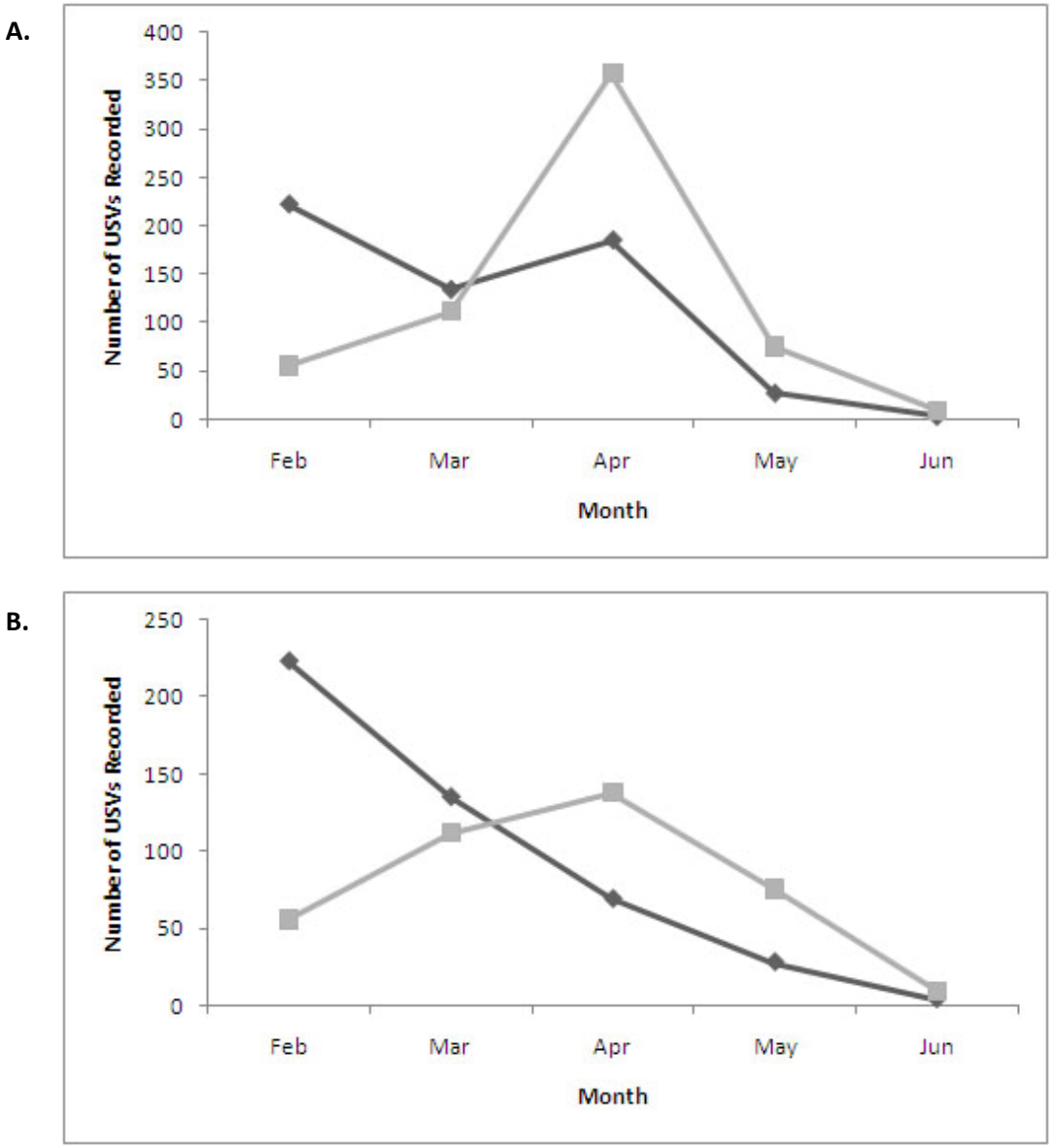


Figure 6. Correlations in USV Production between Species on 3-April.

Vocalizations recorded from free-living *Peromyscus californicus* and *P. boylii* in California in 2008. (A) USVs recorded on 3-April (n = 334) broken down by species and minute when produced, between 8:20 PM and 10:20 PM. This time window covers substantially all of the USV production on 3-April. *P. californicus* is represented by dark gray, and *P. boylii* is represented by light gray. (B) A correlation biplot of *P. boylii* USV production vs. *P. californicus* USV production during each minute on 3-April.

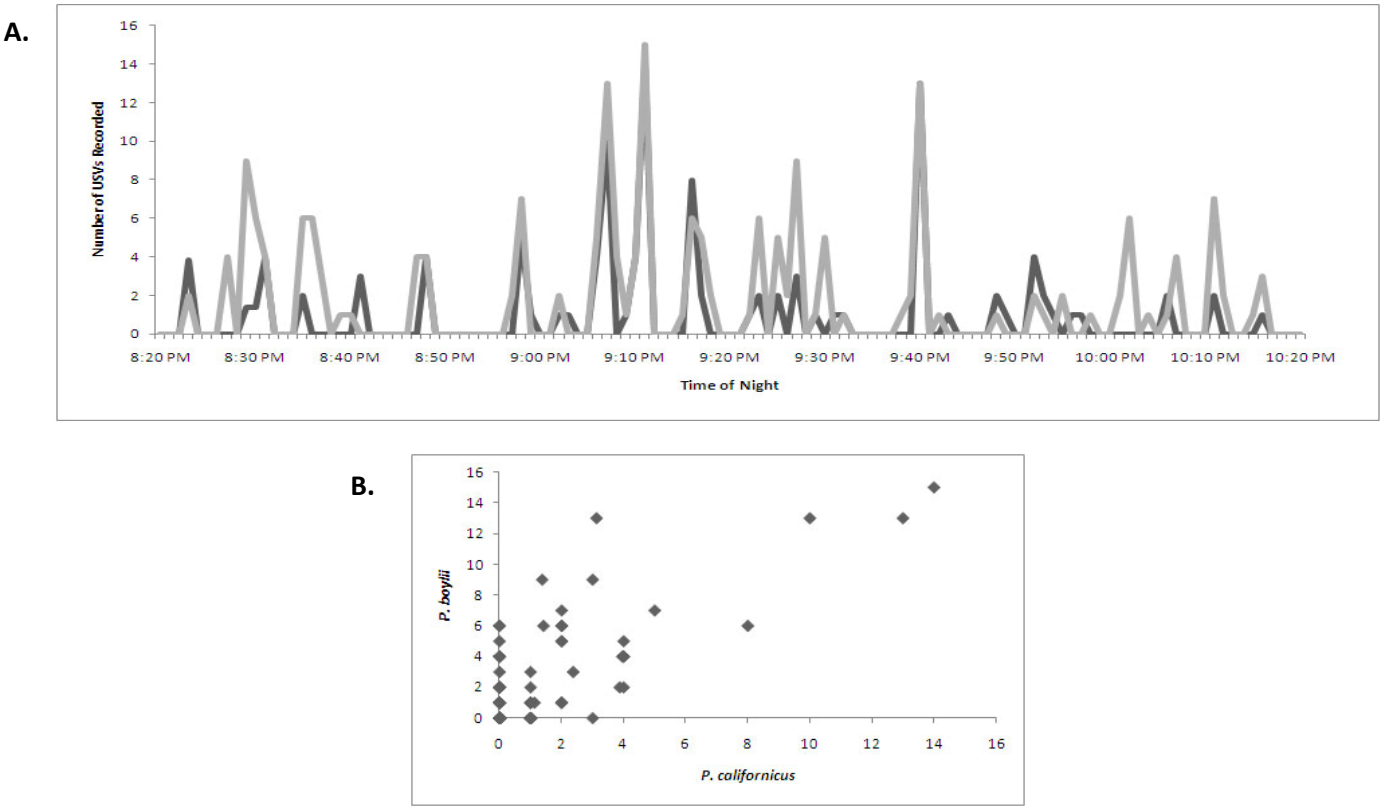
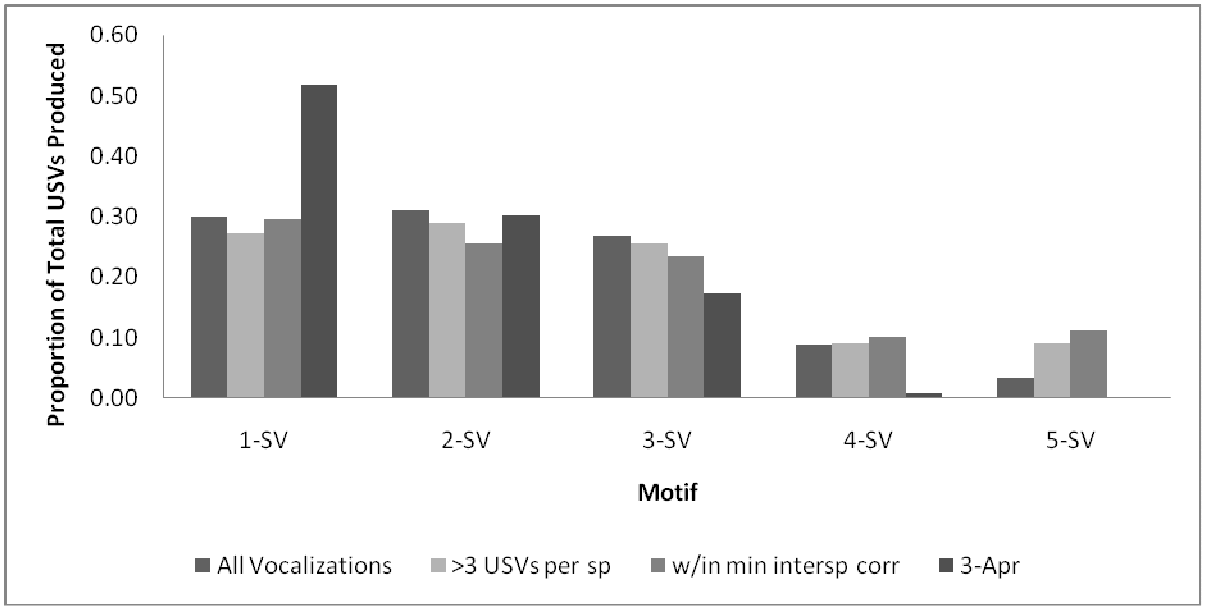


Figure 7. Motif Distribution by *P. californicus*.

Vocalizations recorded from free-living *Peromyscus californicus* in California in 2008. Comparison of *P. californicus* motif distribution within the All Vocalizations dataset, within the nights when both species produced more than 3 USVs each (excluding 3-April), within the nights when significant within-minute interspecific correlations were found (excluding 3-April), and on the outlier night 3-April. 3-April (n=334) was included separately from other nights in order not to skew the data based on the patterns of one night.



APPENDIX B. Signal Strength vs. Distance Linear Regression and Inverse Prediction Interval.

Distances between a detected mouse and the antenna where they were detected were estimated based on the signal strength logged by the telemetry receiver. In order to find the relationship between signal strength and distance, 24 tests were done, where transmitters were placed at known distances (at meter intervals of 1 to 9) from each antenna while the telemetry and logging system were running. Tests were done both at a relatively level area outside of the forest and at one focal area inside the study grid. Tests were replicated three times for each level of treatment: location (barn/grid), telemetry set (A/B), and age (new/old). "Telemetry set" refers to the wires and antennae connected to the receiver. Two of these were used alternately during data collection. "Old" transmitters are those that have been in use >8 days.

Data from these tests was used to make a linear regression of signal strength vs. distance using SPSS. The initial scatterplot of signal strength vs. distance was curved, so the data was transformed in several different ways (variations on taking the log and/or square root of each variable) in an attempt to increase linearity. Regressions were done on the transformations that produced the most linear scatterplots. Among these, histograms of the residual frequency were examined for normality and scatterplots of residuals vs. predicted values were examined to ensure that the residuals were centered relatively closely around zero and the predicted value residuals were between ±2. Of the two transformations that showed the most normal residual histogram and best residual scatterplot, one was chosen based on its slightly higher R^2 and lower standard

error values (both had a p-value of .000). The transformation used for the linear regression was √ (signal strength) vs. √ (distance). From this equation distance could be used to estimate signal strength (y=a+bx, or √signal strength= a+ b√distance) with a known error.

This overall regression was used to determine the proper data transformation. However, ANOVA showed differences in the linear relationship of signal strength to distance based on transmitter (p=.000), for each telemetry set (only one set was used at each focal area) (p=.000), and by age (p=.000). Therefore, individual regression equations – based on the noted data transformation – were found for each transmitter used. Data for these individual regression lines came from distance tests done at each focal area, where transmitters were placed at known distances (each microphone location) from each antenna for three minutes each while the receiver and logging system were running.

After the regression equation for each transmitter was found, an inverse prediction interval was found, in order to determine the error when signal strength was used to estimate distance. This was done by entering all possible signal strength values (they ranged between 100 and 200) into Microsoft Excel, transforming them (taking square root of each signal strength value, or y), using the regression equation to find x (x=(y-a)/b), entering all of these x values into the SPSS spreadsheet containing the test data, and rerunning the regression to get the unstandardized predicted values for y, the standard error for these predicted values, and the confidence and prediction intervals for each predicited value. Using the standard error of prediction formula

$$SE[Pred\{Y|Xo\}] = \sqrt{(\sigma^2+SE[\mu\{Y|Xo\}]^2)}$$

where σ^2 is the standard error of the mean (found on the ANOVA table in SPSS, in the MSE spot), and $SE[\mu\{Y|Xo\}]^2$ is the standard error of the prediction by a particular x (the standard error of the predicted y values given by SPSS), the standard error of prediction (where x finds y) was found. In order to find the standard error of the inverse prediction (where y finds x), the equation

$$SE\ (X') = (SE(Pred\{Y|X'\})/|b|$$

was used, where SE(Pred{Y|X'}) is the standard error of prediction for the estimated x, found by the previous equation for each estimated x.

This standard error was then multiplied by the t-statistic to get the halfwidth of the inverse prediction interval.

Using the x values predicted by the regression equation, the inverse prediction interval halfwidths were added or subtracted from x to get the high and low values of x for each y. The predicted x and high and low x values were then backtransformed (x^2) to get the estimated distance and confidence interval values for each possible signal strength. These calculations were done on an Excel spreadsheet. Both of the preceding equations come from Chapter 7 of the Statistical Sleuth [71]. A summary of the statistical output from the regression model on SPSS 16.0 is included (Fig. B-1).

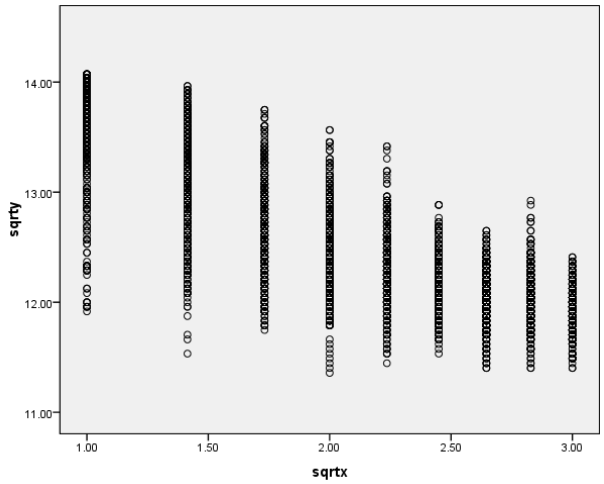
Due to the short height of the mice [72] and the limitations of telemetry receivers with regard to consistent signal detection in discrete areas [73], reliable location of individual mice within the focal area microphone array was problematic. However, the use of the mentioned transmitter tests and signal strength-distance prediction intervals allowed a good understanding of

the telemetry system. Therefore, the assignment of USVs to individuals was possible in many cases.

Figure B-1. Summary of Signal Strength (y) vs. Distance (x) Model Based on Tests of 4 Transmitters.

The following figures and tables summarize the statistical output from the signal strength vs. distance model discussed in Appendix A. Included are: (A) A scatterplot of √signal strength (y) vs. √distance (x); (B) linear regression model summary; (C) Analysis of Variance showing the significance of the model (p>,01); (D) summary of model coefficients showing the significance of distance (p>.01); (E) scatterplot and (F) histogram of residuals; and (G) a test of between-subject effects to show whether variables interact to produce a difference in signal strength.

A.



B.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.794[a]	.630	.630	.37327

a. Predictors: (Constant), sqrtx

b. Dependent Variable: sqrty

Figure B-1, continued. Summary of Signal Strength (y) vs. Distance (x) Model Based on Tests of 4 Transmitters.

C.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1048.402	1	1048.402	7524.444	.000[a]
	Residual	615.154	4415	.139		
	Total	1663.556	4416			

a. Predictors: (Constant), sqrtx

b. Dependent Variable: sqrty

D.

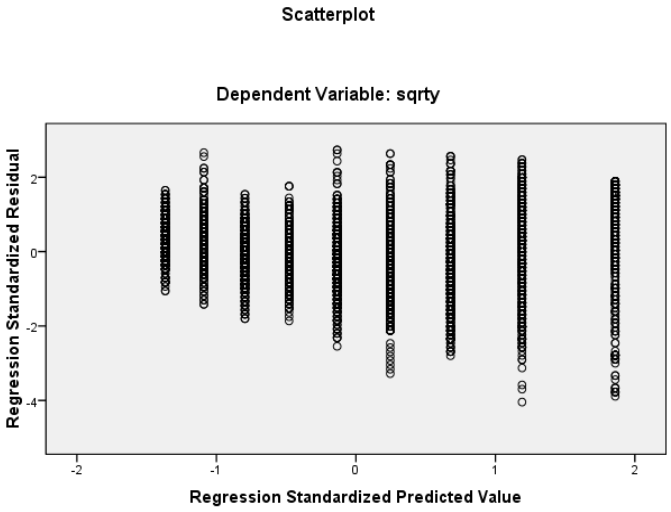
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	14.153	.020		697.364	.000
	sqrtx	-.786	.009	-.794	-86.744	.000

a. Dependent Variable: sqrty

Figure B-1, continued. Summary of Signal Strength (y) vs. Distance (x) Model Based on Tests of 4 Transmitters.

E.



F.

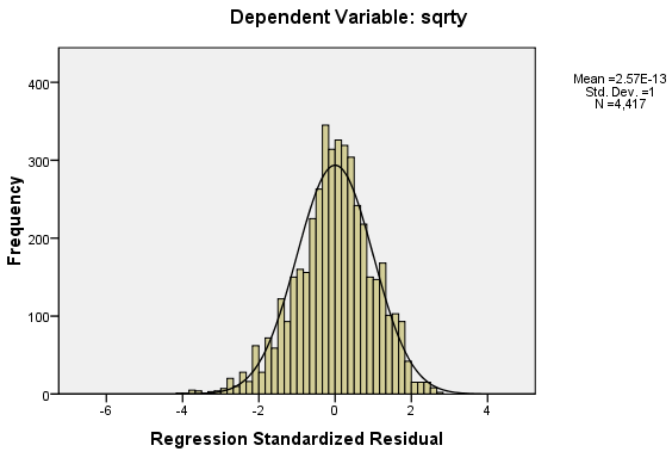


Figure B-1, continued. Summary of Signal Strength (y) vs. Distance (x) Model Based on Tests of 4 Transmitters.

G.

Tests of Between-Subjects Effects

Dependent Variable:sqrty

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1156.478[a]	49	23.602	203.259	.000
Intercept	143240.995	1	143240.995	1233604.875	.000
distance	882.108	1	882.108	7596.798	.000
trans	28.972	3	9.657	83.169	.000
old	7.185	1	7.185	61.881	.000
antenna	10.030	3	3.343	28.795	.000
antenna * distance	.091	3	.030	.260	.854
trans * distance	3.571	3	1.190	10.253	.000
antenna * trans	5.862	9	.651	5.610	.000
trans * old	4.777	2	2.389	20.572	.000
old * distance	1.473	1	1.473	12.687	.000
antenna * old	1.645	3	.548	4.722	.003
trans * old * distance	.984	2	.492	4.239	.014
antenna * trans * distance	4.409	9	.490	4.219	.000
antenna * trans * old * distance	1.703	9	.189	1.629	.101
Error	507.078	4367	.116		
Total	687596.000	4417			
Corrected Total	1663.556	4416			

a. R Squared = .695 (Adjusted R Squared = .692)